BREEDING WHEAT FOR EFFICIENT NITROGEN USE IN LOW-INPUT AND ORGANIC SYSTEMS IN THE PACIFIC NORTHWEST

By

Julie C. Dawson

A dissertation submitted in partial fulfillment of
the requirements for the degree of

DOCTOR OF PHILOSOPHY

WASHINGTON STATE UNIVERSITY
Department of Crop and Soil Sciences

May 2008

To the Faculty of Washington State University:

The members of the Committee appointed to examine the dissertation of JULIE C. DAWSON find it satisfactory and recommend that it be accepted.

_____
Chair

_____

_____

_____

_____

Acknowledgements

I would like to thank my advisor Steve Jones for all the discussions, debates, advice and opportunities over the past four years. He gave me the chance to learn about the research process, to develop professional relationships with scientists from all over the world and to think deeply about some of the most pressing issues of our times. The members of my committee were always willing to help with advice and expertise, and I want to thank each of them for their patience and good humor. I couldn't have asked for a better group of people to work with. Each of them contributed greatly to this project. Dave Huggins worked with me extensively on concepts and measurement of NUE; Leland Glenna provided sociological background and helped design, implement and analyse the survey and roundtable focus groups; Clare Coyne helped with her knowledge of genetic diversity analysis. Eric Zakarison was involved in the project design and interpretation from the first stages to the last and his advice and input were incredibly valuable.

I would like to acknowledge the Winter Wheat Breeding Program technicians and grad students who helped me immensely in this research: Kerry Balow, Meg Gollnick, Steve Lyon, Kevin Murphy, Matt Arterburn, Julia Piaskowski, Lori Hoagland, Janet Matanguihan and Jennifer Moran. Stewart Higgins, Dave Uberagua, Derek Appel, Ron Bolton, Margaret Davies were excellent sources of advice and technical support. Dr. Rich Alldredge was an invaluable resource and I thank him for his clear explanations and his willingness to spend so much time

helping me with the statistical analysis. Dr. Markus Flury provided the LaTeX thesis template file which saved countless hours of formatting. I would also like to thank the faculty, grad students and post-docs in Crop and Soil science for many interesting discussions and for their support. A special thanks to the members of Community Congregational Church, the K-house and the Palouse Food Project for providing motivation and perspective.

I thank Sara and Joe Delong for the opportunity to do on-farm research, and for all their time and expertise in helping with my field plots. Lexi Roach and Jim Moore were very helpful in teaching me about wheat farming in the driest areas of the state and were great fun to work with. Rose Krebill-Prather spent many hours working with me on the design and implementation of the survey, and it would not have been successful without her. Jessica Goldberger and Ray Jussaume in the Community and Rural Sociology department also helped tremendously with the survey. I thank the farmers who took the time to participate in our roundtable discussions and to complete our survey, especially those that helped to organize roundtables and helped refine the survey questions.

This work was funded by the National Science Foundation under a Graduate Research Fellowship and by The Land Institute. Any opinions, findings, and conclusions or recommendations expressed in this material are my own and do not necessarily reflect the views of the National Science Foundation or the Land Institute.

BREEDING WHEAT FOR EFFICIENT NITROGEN USE IN LOW-INPUT AND ORGANIC SYSTEMS IN THE PACIFIC NORTHWEST

Abstract

by Julie C. Dawson, PhD

Washington State University

May 2008

Chair: Stephen S. Jones

This study sought to elucidate the sources of variation for nitrogen use efficiency (NUE) in wheat grown in organic and low-input agricultural systems. Wheat selected under different N regimes was compared for traits related to NUE in the field and in the greenhouse. Annual winter wheat varieties and breeding lines and a perennial bulk population were used in the field study, and annual spring wheat varieties, perennial breeding lines and a series of chromosome addition lines was used in the greenhouse. An analysis of covariance (ANCOVA) and a principal components analysis (PCA) were used to assess variation for traits of interest. From the ANCOVA, it is apparent that there is significant genetic variation for traits related to N use in organic systems in this sample of genotypes. Environmental and

a list of abbreviations and acronyms is found in Appendix A

genotype by environmental interactions are also present. PCA was useful in determining the relationships among measured variables and in grouping genotypes according to their agronomic responses to organic nitrogen management. In addition to the field and greenhouse experiments, the role of participatory plant breeding in meeting the needs of farmers in organic and low-input systems was studied. A mail survey of Washington wheat growers and a series of focus-group roundtables were conducted to obtain input from farmers on the wheat breeding program goals. The survey revealed that 52% of respondents are interested in a participatory wheat breeding program. An analysis of the survey data looked at farmer interest in participatory research to help the breeding program meet its goal of including more farmers in the breeding process.

Table of Contents

List of Tables

List of Figures

Dedication

to my parents, Cathy and Paul Dawson, with love and thanks

Chapter 1

Introduction

Improving crop nitrogen use efficiency (NUE) is important to reducing the environmental impacts of agriculture, for both perennial and annual crops. Most research on NUE has been focused on increasing yield and grain protein per unit of applied nitrogen (N) fertilizer. However, due to increasing concerns about the overuse of synthetic fertilizers, researchers are beginning to look at NUE from the perspective of minimizing N losses and reducing the N required for optimal yields (Cassman et al., 2002). Chapter 2 provides background and discussion on the definitions of NUE in agricultural and ecological systems, effects of genetic and environmental factors, and strategies for improving NUE in organic and low-input agricultural systems.

Organic and conventional systems differ significantly in terms of soil N cycling and traits needed for improved NUE in organic agriculture may be very different than those needed in conventional systems (Watson et al., 2002). To breed crops

with improved NUE in organic systems, breeders must first determine whether there is genetic variation for traits related to NUE and identify varieties with beneficial traits that contribute to NUE. The goals of this study were to explore variation in traits related to NUE among perennial, historic, organically bred and conventionally bred annual wheat genotypes in an organic system.

Historic varieties were developed before synthetic N sources were available, so these varieties may be important sources of adaptive traits for systems with organic N cycling. Foulkes et al. (1998) showed that historic cultivars were better able to extract and use soil N when there was no added fertilizer than modern cultivars were. In perennial wheatgrasses, natural selection has been acting on species in highly competitive prairie ecosystems where N is limited. Deep root systems and a longer period of photosynthesis may mean that perennials are more efficient at capturing and using N. However, it is also possible that modern varieties have important traits for N-uptake because increasing the harvest index (HI) requires the plant to assimilate more N for the same amount of biomass since grain has a higher protein concentration than straw (Sinclair, 1998).

The study presented in Chapter 3 looked at several components of NUE to determine the level of genetic variation for each component and the relative performance of perennial and annual wheat lines. Conducting the field study in an organic system using genetically diverse material provided information about genetic differences that can be used in the breeding programs to select for high NUE under

conditions of relatively low available N. Breeding wheat with superior performance in organic systems will help wheat farmers transition to more sustainable fertility management without economic losses.

A parallel greenhouse study, discussed in Chapter 4 used historic and modern annual spring wheat genotypes, a series of addition lines derived from a cross between Chinese Spring, and annual spring wheat, and *Thinopyrum elongatum*, a perennial wild wheat. The greenhouse study also examined several perennial wheat lines.

Both the field and greenhouse studies were also analyzed through principal components analysis (PCA) in Chapter 5. This was done to explore the relationships among measured variables and among genotypes. Because many of the components of NUE are correlated, either positively or negatively, the use of regression for multivariate analysis is unsatisfactory due to problems with multicollinearity. PCA derives a number of uncorrelated new variables from the original variables so that a more accurate interpretation of correlations and structure in the data is possible (Manly, 1994). This type of analysis was done to explore its utility in characterizing genetic variation for components of a complex trait such as NUE. Knowledge of genetic relationships can be used in preserving genetic diversity, for example among the historic varieties or within the perennial breeding program. Factor loadings and correlations for the measured variables could also be used to determine the amount of redundancy to eliminate variables which are redundant or not well correlated to those variables of interest, making the process of selecting genotypes with high NUE

in organic systems easier.

In addition to the study on NUE, the second half of this thesis deals with the use of participatory methods in plant breeding and research. Participatory plant breeding (PPB) was initially developed because farmers in marginal agricultural areas were not benefitting from formal plant breeding conducted by professional plant breeders (Ceccarelli and Grando, 2007). By involving farmers in the process of selection and evaluation, plant breeders can make their work more relevant. The relevance of PPB to organic and low-input systems in developed and developing countries is discussed in Chapter 6. While some question the utility of participatory plant breeding in the conventional agricultural systems in developed countries (hereafter referred to as developed agricultural systems), a survey of farmers in Eastern Washington revealed that a majority of these farmers would like to be more involved in the research and breeding process. An analysis of the survey data in Chapter 7 explores different characteristics and attitudes of the farmers surveyed to help the wheat breeding program better serve the needs of the growers in this state. Summary data from all the questions on the survey is presented in Appendix C.

A series of grower roundtables was also conducted so that farmers in wheat growing counties in Eastern Washington could sit down with researchers from WSU and have an in-depth discussion of their needs, constraints and future vision of wheat farming in this region. A summary of these discussions and written comments from the survey is included in Chapter 8. These roundtables helped put research on wheat

breeding strategies for low-input systems in context, and farmers participating in the roundtables and in research with the Winter Wheat Breeding program provided substantial input into project goals and directions.

Chapters have been submitted and/or published with multiple authors. Other authors were involved in discussions of the material prior to writing and in making revisions to drafts before submission and during the review process. I conducted the primary literature reviews and experiments and analyses, and was responsible for much of the organization, implementation and analysis of the roundtables and surveys. I would especially like to acknowledge the help of Dr. Huggins in revising Chapter 2, and that of Dr. Goldberger in the analysis of the survey data and the sociological background for Chapter 7. Dr. Glenna contributed greatly to the analysis of the survey data for Chapter 8.

Bibliography

Cassman K.G., Dobermann A., Walters D.T., 2002. Agroecosystems, nitrogen-use efficiency and nitrogen management. Ambio 31(2), 132–140.

Ceccarelli S., Grando S., 2007. Decentralized participatory plant breeding: an example of demand driven research. Euphytica 155, 349–360.

Foulkes M.J., Sylvester-Bradley R., Scott R.K., 1998. Evidence for differences

between winter wheat cultivars in aquisition of soil mineral nitrogen and utilization of applied fertilizer nitrogen. Journal of Agricultural Science 130, 29–44.

Manly B.F.J., 1994. Multivariate Statistical Methods: A Primer, Chapman and Hall, London, chap. Principal Component Analysis. 2 edn., pp. 76–106.

Sinclair T.R., 1998. Historical changes in harvest index and crop nitrogen accumulation. Crop Science 38(3), 638–643.

Watson C.A., Atkinson D., Gosling P., Jackson L.R., Rayns F.W., 2002. Managing soil fertility in organic farming systems. Soil Use and Management 18(S1), 239–247.

Chapter 2

Characterizing nitrogen use efficiency in natural and agricultural ecosystems to improve the performance of cereal crops in low input and organic agricultural systems

Originally published as: Dawson J.C., Huggins D.R., Jones S.S., 2008. Characterizing nitrogen use efficiency in natural and agricultural ecosystems to improve the performance of low input and organic agricultural systems. Field Crops Research 107, 89-101. DOI 10.1016/j.fcr.2008.01.001.

Abstract

Low input and organic farming systems have notable differences in nitrogen (N) sources, cycling and management strategies compared to conventional systems with high inputs of synthetic N fertilizer. In low-input and organic systems, there is

greater reliance on complex rotations including annual and perennial crops, organic N sources, and internal N cycling that more closely mimic natural systems. These differences in farming system practices fundamentally affect N availability and N use efficiency (NUE) and could impact crop traits and breeding strategies required to optimize NUE. We assess genetic and environmental factors that could assist breeders in improving crop performance in low-input and organic farming systems by examining NUE in natural and agricultural ecosystems. Crop plants have often been bred for high N productivity, while plants adapted to low N ecosystems often have lower productivity and higher levels of internal N conservation. Breeders can potentially combine N productivity and N conservation through the use of elite and wild germplasm. Beneficial genetic traits include the ability to maintain photosynthesis and N uptake under N stress and the ability to extract soil N at low concentrations, perhaps through beneficial associations with soil microorganisms. In addition, breeding for specific adaptation to climactic and management practices so that crop uptake patterns match N availability patterns, while minimizing pathways of N loss, will be critical to improving NUE.

Keywords: nutrient cycling; perennial crops; cereal crops

2.1 Introduction

In the initial stages of crop domestication, plants were still subject to many of the same natural selection pressures as their wild progenitors. Environmental heterogeneity and unpredictability were characteristic of these early agricultural systems. Over time, agriculture evolved to overcome production risks associated with environmental variability by developing mechanical, genetic and chemical means to reduce variability and improve crop performance. Today, many agricultural breeding programs are conducted in environments where inputs such as nitrogen (N) fertilizers are highly regulated to ensure that crop deficiencies are minimized. In contrast, low-input and organic farming systems often have limited pools of mineral N and a greater reliance on organic sources and internal cycling of N. Consequently, traits related to nitrogen use efficiency (NUE) in an evolutionary context may be more important for low-input and organic systems than in conventional systems.

Despite its importance, a clear understanding of the major mechanisms and inheritance of NUE is lacking (Basra and Goyal, 2002). Part of this is due to the inherent complexity of NUE, as it is a function of multiple interacting genetic and environmental factors. Disagreements often arise not only in partitioning variation to genetic or environmental causes, but in the denition of NUE itself. Nevertheless, the first stage in many breeding projects is to define the desired phenotype and important traits which can contribute to improvements in NUE.

Genetic mechanisms of agricultural NUE have primarily been studied in conventional systems where traits under selection are often directed toward increasing the response of crop yield and quality to applied N; subject to the law of diminishing returns (Spillman and Lang, 1924). While these systems can create N stress, they do not capture the range of environmental and management factors present in low-input agricultural systems. Here, the range and complexity of environmental stress and interacting genetic components are more similar to less regulated natural ecosystems. Key components of NUE in natural ecosystems relevant to improving NUE in low-input and organic farming systems likely include N conservation, internal N cycling and adaptation to low N conditions. Less is known about the genetic factors controlling N use efficiency in such systems, and whether the genetic mechanisms differ significantly between high and low fertility environments. Therefore, studies on the NUE of plants and populations in natural ecosystems could aid in designing selection regimes and identifying specific traits that are useful for improving varietal performance in low-input and organic systems.

The most immediate goal of improving agricultural NUE is to improve the recovery of N from fertilizer, either organic or synthetic. Globally, only a third of the N in fertilizer applied to cereal crops is harvested in the grain (Raun and Johnson, 1999). Future costs of N fertilizer will increase as natural gas becomes scarcer. Currently, one metric ton of fertilizer N synthesized through the Haber-Bosch process requires $873\,m^3$, or 35 million British Thermal Units (BTU), of natural gas

(Vance, 2001). In addition, transporting and applying such fertilizers takes fuel energy and labor. Many farmers want to minimize costs associated with N fertilizers as well as potential adverse impacts on water, air and soil quality.

Several different strategies are currently being pursued to address problems associated with inefficient agricultural systems and the N cascade (Galloway et al., 2002). Precision N management can improve NUE by tailoring applications of fertilizer N to site-specific conditions in order to reduce N losses and optimize crop performance. Breeding efforts are aimed at developing crop varieties that are more efficient at capturing soil N, thereby decreasing N leaching and denitrification losses and reducing plant N requirements (Cassman et al., 2002). Genetic studies with small grains have been primarily concerned with improving NUE as a means of increasing grain protein content and yield response to applied N fertilizer. While this can also reduce the N requirement of plants for a given yield and protein goal, it does not directly address the environmental impacts of excess N fertilization. The most comprehensive solution is to redesign the cropping system making use of management tools such as rotations, mixtures, and perennial crops. This approach requires the most drastic change but may be necessary when considering agricultural sustainability over a longer timeframe. Many organic and low-input farms use an integrated approach to maximize on-farm nutrient cycling and to build or maintain soil fertility and crop productivity. These systems could benefit from both site-specific N management and crops bred for improved NUE without synthetic N

applications.

Breeding crops specifically for organic and low-input systems is gaining attention as farmers and researchers realize that beneficial traits for these systems may be very different from those that produce high yields in conventional systems (Murphy et al., 2007). Since N is a major limiting factor in low input and organic cereal production, the development of highly efficient varieties could hasten the adoption of these systems and also make it possible to reduce levels of N fertilization in conventional agriculture. Our objectives are to: (1) integrate ecological and agricultural concepts and definitions of NUE; and (2) discuss strategies for breeding grain crops with higher NUE in organic and low-input farming systems.

2.2 Nitrogen Use Efficiency: Concepts and Definitions

2.2.1 NUE of Annual Grains

Moll et al. (1982) defined NUE as the ratio of grain weight to N supply (G_w/N_s) and N supply as the amount of plant available N in the soil. The authors noted that plant available N was difficult to measure and that many researchers substitute applied fertilizer N when calculating NUE. Because all applied fertilizer N is not available to the plant and applied fertilizer N is not the only source of

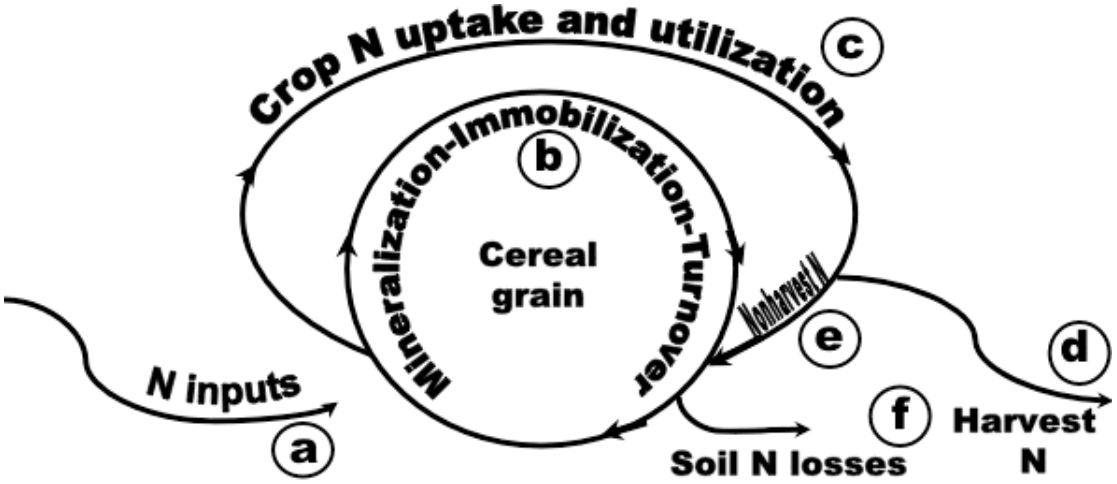


Figure 2.1: Pathways affecting nitrogen use efficiency in cereal cropping systems. Circled letters refer to letters in Table 1.

available N, this definition does not provide a complete picture of NUE. This is particularly the case with low-input and organic systems where inputs of synthetic N fertilizers are minimized or absent. Huggins (1993) adapted the formula of Moll et al. (1982) to aid partitioning between soil and plant physiological process and effects. They redefined N supply as the amount of N potentially available to plants including N losses and immobilization and added the term Nav to represent plant available N. Plant available N was defined as the difference between N supply and N losses associated with leaching, volatilization, runoff, denitrification and immobilization (see Figure 2.1, Table 2.1 and Table 2.2 for a summary of NUE components).

The ratio of plant available N to N supply is the system N retention efficiency (N_{av}/N_s), or the proportion of the N supply that is available to plants that season. While N retention efficiency primarily relates to soil N cycling and flow, it is also

Table 2.1: Components of nitrogen use efficiency for annual crops

Component symbol and descriptor		Example
G_w	grain dry weight	4394 kg ha^{-}1
H_w	harvested biomass dry weight	4904 kg ha^{-}1
N_s	N supply	229 kg N ha^{-}1
N_t	total N in plant	128 kg N ha^{-}1
N_g	N in grain	104 kg N ha^{-}1
N_{av}	plant available N	155 N kg ha^{-}1
G_w/N_s	N use efficiency	19.2 (a, b, c, e, f)
N_t/N_s	N uptake efficiency	0.56 (a, b, c)
G_w/N_{av}	plant available NUE	28.3 (a, b, c, f)
N_{av}/N_s	N retention efficiency	0.68 (a, b, f)
G_w/N_t	N utilization efficiency	34.3 (c)
N_t/N_{av}	available N uptake efficiency	0.83 (a, b, c, f)
N_g/N_s	grain N accumulation efficiency	0.45 9 (a, b, c, d)
N_g/N_t	N harvest index	0.81 (c, d)
N_c/N_t	N carry-over efficiency	n.a.
H_w/N_s	Forage NUE, definition 1	21.4 (a, b, c, e, f)
H_w/N_t	Forage NUE, definition 2	38.3 (c)

After Berendse and Aerts, 1987, Huggins and Pan 2003

The example values are for annual wheat, from Huggins and Pan 1993.

Measurements given in the upper part of the table are used to obtain the example values in the second part of the table. Letters in parentheses refer to pathways in Figure 2.1 that impact the component of NUE being calculated.

Table 2.2: Components of nitrogen use efficiency for perennial species

Component symbol and descriptor	
$MRT * A$	N use efficiency
L_n	relative N requirement
MRT	mean residence time, in days, L_n^{-1}
A	N productivity, g^{-1} N day^{-1}
After Berendse and Aerts, 1987	

related and in part due to plant N uptake. If plants have higher N uptake, there is less soluble N in the soil that could be immobilized or lost (Fiez et al., 1995). This recognizes the complex interaction between plant and soil to determine N availability and uptake. In field experiments, plant available N may be calculated as the total N in plant tissues plus the residual inorganic soil N within the root zone. Using the calculated value of plant available N, it is possible to obtain the plant available NUE (G_w/N_{av}). This is the same as NUE (G_w/N_s) if there are no N losses. Plant available NUE is a measure of efficiency for the plant, whereas the original formula looks at the NUE of the system, without separating plant and soil influences.

It is also possible to measure the grain N accumulation efficiency (GNACE), which is the amount of N in the grain divided by the N supply (N_g/N_s). It serves as a measure of the overall efficiency with which plants extract N from the soil and accumulate it in the grain by harvest. An additional important parameter is the N harvest index (NHI). NHI is the ratio of N present in grain to total plant N content (N_g/N_t), analogous to the harvest index (HI), which is the ratio of grain to total

biomass. It is a measure of N translocation efficiency. Although not directly related to grain weight, it has significance for maximizing grain protein content for a given amount of plant N.

Two plant physiological components, N uptake efficiency and N utilization efficiency, contribute to overall NUE. N utilization efficiency (G_w/N_t) measures the response of grain yield to the total N in the plant. Since total plant N is difficult to measure, most experiments measure aboveground plant N, ignoring the root system. This may not be significant when comparing genotypes that have similar root systems, however, it may be important if comparing plants with different root architecture and biomass. As with the substitution of N fertilizer for N supply, this does not change standard calculation methods but must be acknowledged as a factor when interpreting results. It must also be remembered that NHI, GNACE and N utilization efficiency do not account for potential volatilization losses during translocation. Volatilization is difficult to quantify and reduces total plant N measured at harvest, so if volatilization losses are significant, plants may seem to have greater efficiencies than is actually the case.

N uptake efficiency was defined by Moll et al. (1982) as total aboveground plant N at harvest divided by the total N supply (N_t/N_s). Huggins and Pan (2003) modified this to improve the accuracy of measuring plant efficiency, by accounting for potential losses and calculating plant available N uptake efficiency (N_t/N_{av}). Uptake efficiency is a measure of how much N the plant absorbs in proportion to the

N supply (or plant available N). Plant uptake is closely associated with assimilation, the incorporation of N compounds into plant tissues. Many authors who use the older formula (N_t/N_s) find that uptake efficiency decreases with increasing N supply. Huggins and Pan (2003) found that decreasing efficiency at higher N supply was not mainly due to decreased plant uptake but was mostly because of greater losses from the system. Not accounting for N losses could therefore be misleading when analyzing plant N uptake efficiency.

The definition and components of NUE in annual grains place an emphasis on grain yield and protein. While these two traits are extremely important in agriculture, the definition of NUE as the ratio of grain yield to N supply measures the plant response to available N rather than the efficiency of the system as a whole. In more diverse rotational systems, the NUE of the system over time and the ability to minimize N losses may be just as important as the yield response to N in any particular year. For these systems, studies on the NUE of perennial species in natural ecosystems and pastures is very relevant. From an ecological point of view, the response to available N is not as important as the long-term survival and N balance of populations.

2.2.2 NUE in Natural Ecosystems and Perennial Species

Berendse and Aerts (1987) discussed previous attempts to define NUE in

biologically meaningful ways for natural ecosystems and proposed a new definition that could be useful for assessing adaptation to habitats with different N regimes. They stated that NUE should include two components: (1) the mean residence time (MRT; day) of N in the plant; and (2) the rate of biomass production per unit of plant N (A; g dry biomass g^{-1} N day^{-1}). They defined NUE as the product of MRT and A; the amount of biomass that can be produced per unit of N obtained by the plant (g dry biomass g^{-1} N). The MRT is the average length of time a molecule of N remains in living plant tissue and is dependent on the rate of N loss from the plant through volatilization, root exudation, herbivory and tissue senescence. Species with a long MRT (low N loss rate) would be favored in environments with poor N supply, whereas species with high A would be favored in comparatively N-rich environments. This definition of NUE has also been applied to perennial forages (Vazquez de Aldana and Berendse, 1997). Forage NUE, however, is often measured as the amount of forage dry matter produced per unit of applied N (Zemenchik and Albrecht, 2002). This is analogous to the measurement of grain yield per unit of applied N in annual cereals, but as previously discussed does not account for N losses and immobilization, or the availability of N from other sources.

2.2.3 Unifying Definitions of NUE

While the terminology is different, there are clear similarities between the definition of NUE in agricultural systems and that in natural ecosystems. Both

measure biomass produced per unit N, although in natural ecosystems the emphasis is on total biomass, whereas in agricultural systems the harvested biomass (grain or forage yield) is a major consideration. In annual cropping systems, NUE calculations are largely based on measurements at harvest as this is the end of the plant's life-cycle. However, calculating NUE at a single point in time rather than at several different growth stages may not give a complete picture of the N dynamics of the crop over the growing season. If certain varieties have lower levels of N loss, they may be able to produce biomass with less total N over their lifecycle than varieties that have more rapid N turnover, even if they have equivalent yield and N concentration at harvest.

In natural and agricultural systems with biannual and perennial species, biomass production and internal plant N turnover over multiple seasons becomes an increasingly important factor of NUE. The yearly carry-over of N in roots, crowns and other living tissues of perennial species constitute N utilization directed towards important post-harvest physiological functions such as plant re-growth and winter survival.

Therefore, in addition to N utilization efficiencies of annual crops that evaluate harvested biomass (G_w/N_t) and N (N_g/N_t), we propose to add N utilization efficiency components that assess plant N carry-over (N_c) into the next season by biannual or perennial species. Here, N_c is defined as the amount of plant N in living tissues at harvest which along with N in non-living tissue (straw, roots) and N in the

grain (N_g) comprises total plant N (N_t). The N carry-over efficiency can then be defined as N_c/N_t and the contribution of N_c towards N utilization efficiency (G_w/N_t or H_w/N_t, where H_w is the dry weight of any biomass that is harvested) can be expressed as two components: $(H_w/N_c)(N_c/N_t)$. Similarly, the NHI (N_g/N_t) can be partitioned into meaningful components where NHI = $(N_g/N_c)(N_c/N_t)$. Here, N_g/N_c is an N partitioning component related to reproductive and survival strategies of the plant where N resources are allocated to protein synthesis in seeds and/or to physiologically active non-reproductive tissues such as leaves, crowns and roots.

The inclusion of N_c in the evaluation of NUE uses a mass balance approach to address issues raised by N MRT. But rather than assuming a steady-state condition in plant (or population) N retention has been reached to enable the calculation of MRT, critical time periods as defined by key physiological or management criteria allow NUE assessment to be linked to the dynamics of system N cycling and flow. Consequently, although harvest is often a critical time period for evaluating NUE, other physiologically important stages may be identified and used as selection criteria in a breeding program or to evaluate different N management strategies. Evaluating key NUE components at strategic times and integrating this information over the course of a crop sequence or rotation can provide the basis for assessing NUE over a wide variety of agricultural systems using the same definitions and framework.

2.3 Genetic and Environmental Variation in NUE

Previous studies have extensively characterized crop performance (e.g. yield, quality, NUE) under various fertilizer and management regimes in different environments. Results are commonly inconsistent and disagreements arise when partitioning variation in crop performance to genetic or environmental factors. Conflicting results could be due to differing research methods, management practices, climate, genetic materials, and definitions of NUE (Van Sanford and MacKown, 1987; Huggins and Pan, 2003). For example, studies involving a narrow subset of elite genetic material are less likely to find significant genotypic variation for NUE than studies that sample a diverse group of modern and historic varieties. In addition, soil N supply, total plant N, harvested N and N lost from the system are variables that are temporally and spatially dynamic and difficult to accurately measure, therefore making comparisons among studies in different environments problematic (Fowler et al., 1990).

Despite the variation in crop performance arising from genetics, environment and their interaction, studies of forages and annual grains over a wide range of N supply show that agricultural NUE increases as N fertility decreases (Dhugga and Waines, 1989; Huggins, 1991; Gauer et al., 1992; Huggins, 1993; Oritz-Monasterio et al., 1997; Vazquez de Aldana and Berendse, 1997; Jiang and Hull, 1998; Jiang et al., 2000; Singh and Arora, 2001; Zemenchik and Albrecht, 2002; Huggins and Pan,

2003). Reductions in NUE with increasing N supply could result from reductions in any of the components, including N-uptake efficiency, N utilization efficiency and N retention efficiency. Studies on wheat and perennial grasses have shown reductions in all of these components (Cox et al., 1986; Dhugga and Waines, 1989; Huggins and Pan, 2003; Jiang et al., 2000; Morris and Paulsen, 1985; Oritz-Monasterio et al., 1997). For example, Oritz-Monasterio et al. (1997) found that in all varieties evaluated, both uptake and translocation/utilization efficiency were reduced at higher N supplies, causing an overall reduction in NUE. Morris and Paulsen (1985) and Cox et al. (1986) showed a reduction in translocation efficiency at high N compared with low N supplies. Dhugga and Waines (1989) attributed decreased NUE at high N to higher volatilization losses because the plant was unable to assimilate all the N taken up. Huggins and Pan (2003), in contrast, found that there actually was a slight increase in available N uptake at higher N levels, but there was a severe decrease in soil N retention efficiency which resulted in a net reduction in NUE.

Studies examining post-anthesis N nutrition of wheat in order to enhance grain protein provide an interesting example of the interactive effects of genetics and environment. Many farmers increase the rate of N fertilization to meet grain protein concentration targets when growing high-yielding varieties of wheat. Available N early in the season generally contributes to increasing vegetative growth and establishing the reproductive sink capacity, which determines the maximum possible yield. However, excessive vegetative growth can use up available soil water in drier

areas and restrict grain production later in the season (Halvorson et al., 2004). Applying high rates of synthetic N at early growth stages may lead to high losses since plant demand is initially low. Loss of N from available pools, however, is dependent on the strength of competing N pathways including leaching, volatilization and immobilization from the time of application to N uptake. Consequently, synchronization of N application with crop N demand may not lead to greater NUE, rather it is the synchronization of N availability with plant N demand and uptake coupled with the lack of synchronization of available N with competing N pathways that promotes greater NUE. Post-anthesis N fertilization of wheat can provide N for grain protein accumulation, but may not be effective if foliar N uptake is limited, insufficient soil moisture restricts root uptake, or competing N loss pathways reduce N availability (Cox et al., 1985a; Harper et al., 1987; Soon, 1988). Lack of available N uptake due to environmental or physiologic constraints can occur even when the sink strength for grain protein synthesis is high, resulting in N losses and low NUE.

Plants with more extensive root systems may be able to reach moisture and N stored lower in the soil profile. Cox et al. (1985a) found a significant negative relationship between N assimilation after anthesis and total aboveground dry matter at anthesis and attributed some of this variation to certain genotypes having larger root systems or root systems that used soil N more efficiently. Their interpretation was that lines with high aboveground biomass at anthesis probably had less biomass belowground in roots, and were not able to take up N later in the growing season at

lower concentrations and greater depths in the soil. McKendry et al. (1995) found that the two best varieties for NUE were better able to survive extreme drought, suggesting more extensive root systems. Similarly, in perennial grasses grown under drought conditions, species and varieties with more extensive root systems had greater N uptake (Jiang et al., 2000; Zemenchik and Albrecht, 2002).

Organic and low-input systems usually have very different seasonal N cycling and availability than conventional systems that use synthetic fertilizers. Reliance on organic N sources requires an understanding of organic N mineralization-immobilization and turnover (MIT) patterns in relation to crop N demands and N loss pathways. MIT is stimulated by temperature and water conditions that often favor plant growth and biological regulation of N availability and assimilation by plants can be augmented in these systems. For example, the use of perennial species and more diverse crop rotations can increase the temporal and spatial regime of active root systems, thereby limiting N losses and increasing NUE (Collins and Allinson, 2004).

Studies on NUE in annual wheat have established that significant genetic variation exists for traits related to NUE (Dubois and Fossati, 1981; Loffler and Busch, 1982; Paccaud et al., 1985; Van Sanford and MacKown, 1986; Fowler et al., 1990; May et al., 1991; Singh and Arora, 2001). In addition, the heritability of these traits is high (Davis et al., 1961; Loffler and Busch, 1982; Fowler et al., 1990; Cox et al., 1985b; Coque and Gallais, 2006; Agrama et al., 1999; Presterl et al., 2002). In

wheat, significant genetic variation exists for total N uptake (McKendry et al., 1995) and for translocation efficiency (Johnson et al., 1968; Cox et al., 1986). Genetic variability for post-anthesis N uptake has also been reported (Austin et al., 1977; Neales et al., 1963; Clarke et al., 1990). N uptake and N remobilization also appear to be independently inherited traits so favorable alleles could be combined when breeding for NUE (McKendry et al., 1995; Johnson et al., 1967; Sattlemacher et al., 1994; Presterl et al., 2002). In Kentucky bluegrass, a perennial species, there are significant differences between varieties for N uptake and NUE (Jiang and Hull, 1998; Wilkins et al., 1999). The presence of genetic variation for traits that contribute to NUE suggests that breeding for improved NUE in low input and organic systems is possible.

It would be useful to have more information on the relative importance of many traits related to improved NUE, however, the environmental context and the particular genotypes grown appear to have a large effect on research findings. The genotype by environment (GxE) interaction may have a larger effect on NUE than the genotypic contribution itself (Bertin and Gallais, 2000). High levels of GxE interaction mean that it is important to conduct research in the target system, whether the goal is to understand factors that contribute to NUE in that system or to breed varieties with high NUE. This is especially important in low-input and organic systems which are characterized by environmental heterogeneity and which cannot be made uniform through the addition of fertilizer N.

2.4 Ecological Effects of N Limitation

The efficient use of N should be favored by natural selection, and may have been under significant selection pressure during the domestication and evolution of crop species. Directional selection, whether natural or artificial, is expected to reduce the amount of genetic variation present in populations (Byers, 2005; Ackerly et al., 2000). However, environmental heterogeneity over time or space could lead to the maintenance of genetic variation at loci that contribute to NUE. Environmental unpredictability can lead to selection for phenotypic plasticity and the ability for plants to produce more than one phenotype depending on the environmental conditions (Byers, 2005; Górny, 2001).

NUE in terms of evolutionary fitness is different from NUE in an agricultural setting. In natural ecosystems, inorganic N pools are often extremely limited, and natural selection may work to maximize the conservation of N within plant tissues rather than the maximum biomass production per unit N (Vazquez de Aldana and Berendse, 1997; Silla and Escudero, 2004). There appears to be an ecological trade-off between N productivity and MRT. In fertile environments, a high relative growth rate gives species an advantage, but also means higher rates of nutrient turnover and loss from plant tissues (Berendse and Aerts, 1987; Aerts and van der Peijl, 1993). In low N environments, species with a high growth rate will produce more biomass in the short term, but will have lower productive potential after a few

seasons compared to species adapted to low N environments which have a longer MRT and lower rates of N loss (Aerts and van der Peijl, 1993). Early successional species, often annuals, may have an advantage when colonizing new areas because of their rapid growth rate, but then are displaced by species with slower growth rates and better internal N conservation (Tilman, 1986).

Adaptation to low N environments includes a higher root to shoot ratio and a lower relative growth rate. Greater root biomass and mycorrhizal connections are more important than a rapid absorption capacity since mass flow and diffusion through the soil limits the rate at which N is available (Chapin, 1980). Because root absorption rates are proportional to efflux rates, reducing the absorption rate also limits nutrient loss. Species adapted to fertile environments have higher rates of fine root turnover, which increases the absorption capacity per unit of root length, but also increases nutrient loss because resorption of N from senescing roots is minimal (Silla and Escudero, 2004). Species adapted to low N conditions are able to take up more N at low concentrations than those adapted to high N conditions and vice-versa (Chapin, 1980; Jiang and Hull, 1998). In a study of perennial grass species adapted to, or bred for, different environments, species adapted to environments with nutrient limitations had low internal N turnover, and low biomass production per unit N. Species bred for nutrient rich habitats, such as pasture grasses, had high internal N turnover in both low and high fertility treatments and had high biomass production under high N conditions, but produced significantly less biomass in

low-fertility soils (Vazquez de Aldana and Berendse, 1997). Since most crop plants were selected in, and for, nutrient rich environments, genes from native grasses or other wild relatives adapted to low N environments may be useful sources of genes for increasing N uptake and NUE in nutrient limited environments.

In perennials, N stress leads to lower rates of leaf turnover, higher root biomass, fewer tillers and less resource allocation to reproduction. This is in contrast to annuals that put all their resources into reproduction when faced with high stress levels (Chapin, 1980). Species with rapid growth rates usually have high leaf N concentrations and photosynthetic capacity, but shorter leaf life span (Silla and Escudero, 2004). Under nutrient limited conditions, slower growing species have a greater ability to maintain higher leaf N concentrations for photosynthesis (Chapin, 1980). This is because when N becomes available, plants take up more than is necessary to support the biomass they produce, often called luxury consumption. This extra N is then used for later growth, rather than being put into a flush of biomass production that can lead to plant N stress under subsequent conditions of N limitation (Chapin, 1980). This is the reverse of what would be expected in an agricultural setting, where biomass and yield response to added N is a key selection criteria, and luxury consumption to increase grain protein is achieved through adding more N than is necessary for maximum yield.

2.5 Management Effects on Nitrogen Use Efficiency

2.5.1 Nitrogen from Plant Sources

Organic systems differ from conventional systems in many ways, including the use of biological mechanisms of fertility and pest control and the inclusion of diverse rotations to balance on-farm nutrient cycling. There have been many studies on the role of green manures and cover crops in providing and retaining N in organic systems. In conventional systems, wheat yields are often higher following legumes than following another cereal (Soon et al., 2001; Strong et al., 1986; Stopes et al., 1996; Weston et al., 2002; Dalal et al., 1998; Huggins, 1991), and the N uptake of a wheat crop is greater following legumes than that of wheat following wheat or fallow (Soon et al., 2001; Huggins, 1991; Badaruddin and Meyer, 1994; Campbell et al., 1990). Grain protein content and concentration is also greater following a legume crop (Strong et al., 1986; Badaruddin and Meyer, 1994; Campbell et al., 1990; Biederbeck et al., 1996). There are several reasons why rotations may improve the N dynamics of agricultural systems. Huggins (1991) attributed the increase in wheat yields after Austrian winter pea either to the peas supplying greater N for plant uptake or increasing the N uptake efficiency of the wheat crop. A break crop can also reduce the incidence of soil-borne cereal diseases. Controlling diseases leads to more

vigorous plants, which may increase N uptake efficiencies because of healthier roots and greater density of root hairs (Cook et al., 1987).

Crop rotations are a fundamental component of organic systems, and legume crops are often planted to enhance nutrient cycling and availability to other crops in the rotation. Crops with high N demands are usually grown after a green manure or legume crop. Although there is some rotational benefit from grain legumes, it is unlikely that much N is provided to the subsequent crop. Dry beans harvested for grain may fix up to 200 kg N $ha^{-1}yr^{1-1}$ but most of this is removed at harvest. Grain legumes do conserve soil N by fixing atmospheric N, thereby leaving residual N in place for the next crop (Soon et al., 2001). However, only 50% of the total N in grain legumes is from biological fixation, in comparison to up to 80% in forage legumes (Watson et al., 2002).

The inclusion of a legume crop decreases reliance on external fertilizer if the legume crop supplies a significant proportion of the N for the next crop. Cover crops or green manures not harvested for grain are more effective than grain legumes at providing N to the subsequent crop. Soon et al. (2001) found that more N is mineralized from cover crop residues than from wheat or field pea residues. In a study comparing different legumes in rotation with wheat, only rotations that included a legume green manure crop had a positive N balance during two cycles of the rotation (Soon and Clayton, 2003). Another UK study showed that winter wheat yields were greater after legume green manures than after trefoil or ryegrass green

manures, and that only the wheat following clover green manures had high enough grain protein for bread flour (Stopes et al., 1996).

In areas of high rainfall, legume green manures can produce more soluble N than the subsequent cereal crop is capable of taking up during its early growth period, increasing the risk of leaching. A pea green manure in the UK provided 335 kg N ha^{-1}, but only 13.3 kg N ha^{-1} was recovered in the following winter barley crop, probably due to low release rates from the incorporated plant material. The authors estimated that denitrification and leaching losses were small, but may have contributed to the poor crop N recovery (Redman et al., 1989). A study in Sweden with different pasture compositions and management found that green manure crops, particularly clovers, contributed much more N to the system than pastures mowed for forage production (Torstensson, 1998). Green manure treatments contained 170 kg N ha^{-1}, which was almost three times the N in mowed plots. In comparison, there were only 28 kg N ha^{-1} in barley residues. A study in Scotland had similar results, with a white clover green manure providing the highest levels of nitrate to the subsequent crop (Baggs et al., 2000). Mixed clovers and grasses fixed up to 250 kg N ha^{-1} each year, depending on the percentage of clover in the planting (Baggs et al., 2000).

In dry areas, studies have found substantially less net N mineralization than areas with higher rainfall and warmer temperatures. A study in Saskatchewan, Canada, found an average net mineralization of 18 kg N ha^{-1} for four different green manures tested in a wheat-based cropping system, with the highest being 38 kg N

ha^{-1} for chickling vetch and black lentil (Biederbeck et al., 1996). In Queensland, Australia, an annual medic green manure provided from 10 to over 30 kg N ha^{-1} mineralizable N to the following wheat crop, but wheat yields following medic were still higher than those of continuous wheat with 50 kg N ha^{-1} synthetic fertilizer (Watson et al., 2002). In Northern Idaho, pea green manure provided 94 kg N ha^{-1}, while harvested seed peas provided 86 kg N ha^{-1} and summer fallow provided 73 kg N ha^{-1} fertilizer N equivalent, calculated based the difference in yield between wheat grown after the legume or fallow and that of wheat following spring barley. Yield of a winter wheat crop following the pea green manure was similar to that of wheat following fallow, but in the fallowed soil, organic matter was lost to mineralization (Mahler and Auld, 1989).

Cover crops are often grown to minimize leaching and soil erosion over winter and are taken out before planting a spring crop. Subsequent mineralization can provide N to the next crop, but synchronizing the release of N from residues with the periods of strong crop demand is difficult. Mineralization of N from legume residues may match wheat N demand fairly well, increasing uptake and decreasing the potential for leaching (Campbell et al., 1990; Badaruddin and Meyer, 1994). Rotations appear to improve N availability and uptake, and lower residual soil nitrate concentrations have been reported in wheat following legumes than continuous wheat (Soon et al., 2001; Soon and Clayton, 2003). Non-legume green manure crops can also increase system NUE, primarily by immobilizing N to limit

over winter leaching followed by mineralization of this N and use by the crop after the green manure (Watson et al., 2002). Other studies, however, have shown that green manures can result in excess N which is subsequently lost, or can immobilize soil N, reducing its availability during the growing season. Kramer et al. (2002) found that N from vetch residues did not become available until 70 days after planting a maize crop. In comparison with conventional fertilizer, vetch N was released and taken up at a more constant rate over the season, while conventional fertilizer N was more available at the beginning of the season, but decreased in availability as the season went on. Despite differences in timing, however, there was no difference in crop N uptake efficiency (calculated as total aboveground plant N/ N applied) between the conventional and low-input (vetch) N sources.

The C:N ratio of residues has a large effect on decomposition and mineralization rates. Most legume residues have relatively low C:N ratios, generally between 12 and 25 (Torstensson, 1998). In contrast, cereal residues may have a C:N ratio of 80 or more, which can lead to net immobilization of N for months or years after incorporation. In general, there is no net mineralization in the first season from residues with a C:N ratio greater than 25. Incorporation of wheat residues will most likely reduce the amount of immediately available N, but will increase the rate of straw decomposition and eventual release of N compared to leaving straw on the surface (Schoenau and Campbell, 1996). In contrast, incorporation of legumes can release about $150\,\mathrm{kg\,N\,ha^{-1}}$ over 3 months (Berry et al., 2002). Cereal straw only

contains about 35 kg N ha^{-1} compared with up to 150 kg N ha^{-1} for some vegetable residues. This has led some authors to suggest that including crops with a broad range of C:N ratios can help cycle and conserve N within the cropping system and can increase the soil capacity to supply N as needed for growing crops (Watson et al., 2002).

2.5.2 Nitrogen from Animal Sources

Animal manures are an important source of N in many integrated organic and low input farming systems. Fresh manure has lower C:N ratios than composted manure, but is more difficult to store and transport if it is not from on-farm livestock. There is evidence that at least 50% of manure N is lost in storage and transport and another 25% is lost after application (Bouldin et al., 1984). Composted manures cause N to be held in more stable forms and are easier to store and transport, but because of the increased stability composting causes a significant decrease in short-term N availability. An incubation study by Tyson and Cabrera (1993) with composted poultry manure showed a gradual release of inorganic N, mineralizing 0.4-5.8% of the total N over 56 days compared with 25.4-39.8% of total N in uncomposted poultry manure. Despite lower N availability, composted manures have other benefits such as increasing soil pH to alleviate soil acidification from synthetic fertilizers that release inorganic N as ammonia (Tyson and Cabrera, 1993). Rather than being an immediate source of plant-available nutrients, composted

manure has a longer-term role in building soil organic matter and stimulating microbial activity (Watson et al., 2002).

One of the primary differences between organic and conventional N fertilizers is the release rate of inorganic N compounds. The capacity for plants to take up organic molecules containing N such as amino acids has been demonstrated, but is not yet well understood. Genotypic differences exist in wheat cultivars for the capacity to take up amino acids, and this may affect their performance in organic systems (Jennifer Reeve, unpublished data 2007). Despite this capacity, the majority of plant N uptake is likely to be inorganic N, so mineralization rates and timing of N availability from organic fertilizers is critical to understanding the NUE of cereals grown under organic management practices. Standard inorganic soil N tests are less useful for evaluating N availability in farming systems that rely on N derived from organic amendments (Watson et al., 2002). Organic systems usually do not have high levels of mineral N in the soil profile (Power and Doran, 1984), but often have greater mineralization potential due to higher levels of soil organic matter (Stockdale et al., 2002; Drinkwater et al., 1998).

2.5.3 Tillage Regime

Soil disturbance and incorporation of surface residues and plant biomass have a major affect on N availability. Soil disturbance from mechanical tillage stimulates

soil microbial activity which increases soil MIT and N availability (Watson et al., 2002). Increasing soil moisture after a prolonged dry spell, or thawing after freezing conditions also results in a flush of N mineralization (Cassman et al., 2002). When green manure crops are used as a source of N, incorporation is often used to accelerate the mineralization of organic N. Reliance on tillage, however, results in higher erosion risk. Reduced tillage or no-till management can minimize soil erosion, but can have both positive and negative impacts on N availability. For example, crop residues help prevent runoff, which reduces N losses, but surface residues can also immobilize N decreasing its availablity to the crop. Eliminating tillage often results in decreased nitrate concentrations in the soil profile compared to conventional tillage. Soon and Clayton (2003) reported residual nitrate levels two times greater in conventional tillage than no-till for the top 120 cm of soil. Increased N immobilization, leaching and denitrification in no-till systems can contribute to this phenomena (Schoenau and Campbell, 1996).

Although tillage affects N mineralization and availability, Soon and Clayton (2002) found that tillage regime did not have a significant effect on total crop N content. Similarly, in a study in the Palouse region of Washington, total aboveground N at maturity was the same for both conventional and no-till wheat, despite lower available N in no-till (Huggins, 1991). Reduced mineralization rates in no-till could limit grain yield if there were significant N deficiencies. This may be more critical in organic and low input systems that depend on organic fertilizers and crop residues

for crop nutritional requirements. In a study of barley and pea intercropping, the only treatment with net mineralization was incorporated pea residues. Mixed barley and pea residues and barley residues alone resulted in N immobilization, regardless of tillage, and pea residues alone without incorporation resulted in net immobilization (Hauggaard-Nielsen et al., 2003). Dou et al. (1994) compared corn yields following a winter green manure killed using herbicides or tillage. Corn grown with conventional tillage did not suffer from N deficiencies, however, in the no-till herbicide-killed treatment there was significantly less N accumulation and corn yields responded to N fertilization. In addition, no-till corn had severe weed competition during the growing season which the authors attributed to lack of tillage for control. The N mineralization rates were very different for the two systems. In the conventional tillage system, there was rapid increase in mineralization rates during the first month, followed by a slower increase for the rest of the growing season. In the no-till system, there was a more gradual increase in mineralization rates during the first two months and then a leveling off of the mineralization rate. The study did not examine mineralization the following year or the total net mineralization in the two systems.

A mixed tillage organic system provided comparable yields to a conventional, tilled system in a study done in Pennsylvania (Drinkwater et al., 2000). Study comparisons included a series of tillage treatments in conventional and organic systems. No-till organic methods produced very poor yields, partially because the green manure vetch did not germinate well in high residue conditions and only

provided 75% of the amount of N provided by the vetch green manure in mixed-tillage system. Primary tillage caused a large amount of N mineralization, even though the total N produced was equivalent to the no-till mowed vetch. Management of the green manure with a chisel and disc was the only treatment that provided acceptable yields for the organic system while keeping soil mineral N pools at lower levels to prevent N losses. Cultivation for weed control in the organic systems also increased control of the timing of N mineralization. In an organic system, tillage and mid-season mechanical weed control may provide a needed N boost to growing crops, but could cause N leaching or denitrification if not taken up by the crop (Watson et al., 2002). The challenge is to time tillage so that it stimulates mineralization when the growing crop needs N, but so that it does not result in N losses from the system or excessive erosion. The effects of tillage on N cycling must also be balanced with the need for mechanical weed and disease control at certain points of the growing season, which may or may not correspond to the optimal time to stimulate N mineralization. This is an area that needs much more research if reduced tillage organic systems are to succeed.

2.6 Strategies for Improving NUE

Cereal grain yields are often lower in organic than conventional systems, and problems with synchronizing crop demand for N with N mineralization explains part

of this yield gap (Watson et al., 2002). Another portion of the yield gap may be because the cultivars that are compared in conventional and organic systems have been bred for conventional systems and thus are not adapted to organic conditions. Interestingly, the yield reduction sometimes observed when crops are grown organically is greater for crops that have had extensive modern breeding, such as wheat and barley, than for crops that have had relatively little breeding, such as oats or triticale (Watson et al., 2002). Even when varieties are tested under conditions of no added fertilizer N, this does not accurately represent soil and fertility conditions on organic farms, therefore, varieties selected with modern conventional breeding methods are unlikely to have optimal traits for organically managed systems. One particular difference between organic agriculture now and that before 1950 is that high-yielding crops such as hybrid corn have been developed which have a strong demand for N over a short time period, and this may not be easily compatible with organic fertilizers. This may be partly overcome by growing crops that have lower levels of N uptake and no sharp peaks in demand, such as wheat (Pang and Letey, 2000). It is also critical to breed crops that have the same high yield potential and are adapted to organic fertilizers and management practices. Adaptation to organic systems will require a suite of traits including nutrient use efficiency, durable disease resistance, competitive and allelopathic characteristics for weed suppression and quality and nutritional characteristics. This paper focuses specifically on the improvement of NUE, while recognizing the potential interactions among desirable

traits in low input and organic systems. Other traits are discussed in, for example Ceccarelli (1996), Lammerts van Bureren et al. (2002), Mason and Spaner (2006) and Murphy et al. (2007).

2.6.1 Maintaining Photosynthesis under N Stress

Increasing the ability of leaves to continue photosynthesis under N stress is a potential avenue for improving NUE in agriculture. Increasing leaf production can increase the N recovery of perennial grasses, especially in the second year (Zemenchik and Albrecht, 2002). Perennials subjected to nutrient stress may rely more on N remobilization for leaf production. In an experiment with perennial grasses, new leaves contained 52% translocated N under low N conditions, compared to only 12% translocated N in plants growing under high N conditions, although the total amount of N translocated in the two treatments was similar (Li et al., 1992). In annual grains, the size and duration of active leaf tissue affects N uptake and the amount of plant N available for remobilization. Plants that do not senesce their leaves until very late have a greater capacity to take up N during grain fill because continued leaf activity promotes the uptake of soil N (Woodruff, 1972). Stay-green plants delay leaf senescence during grain filling, and may still have active leaf tissue when the grain is completely mature.

There may be a trade-off, however, between the efficient use of N for

photosynthesis and leaf longevity. In short lived leaves, most N is allocated to photosynthetic structures and enzymes, while in longer-lived leaves it is necessary for the plant to invest in defensive compounds (Hikosaka, 2004). This trade-off may be more apparent when comparing different species rather than varieties within a species. In winter wheat, additional plant available N delayed leaf senescence and increased the amount of vegetative N which could then be translocated to the grain (Spiertz and Ellen, 1978). In some stay-green genotypes of maize, longer maintenance of leaf chlorophyll resulted in a 10-12% increase in grain weight (Spano et al., 2003). A study by Duncan et al. (1981) found that stay-green lines of sorghum had a greater leaf area duration and greater chlorophyll content than senescent types. Therefore, they may have greater photosynthetic capability which led to greater potential biomass and grain yield. Stay-green lines also established their adventitious root system earlier and always had greater root density than senescent lines. Also in sorghum, almost 70% of the total variation in grain yield was explained by total plant N content, and greater leaf N content may have allowed stay-green varieties to continue photosynthesis and N uptake under drought stress while senescent varieties had to rely on N and photosynthate translocated from the leaves and other tissues (Borrell and Hammer, 2000).

The interactions between N availability, late-season uptake, and remobilization are complex. Roots need a supply of photosynthate to absorb soil N, so late season photosynthesis helps maintain N uptake after anthesis (Kihlman-Falk, 1961).

However, soil N deficiencies can also lead to early senescence because developing grain requires N that the plant supplies through remobilization of vegetative N (Sattlemacher et al., 1994; Lafitte and Edmeades, 1994; Spiertz and Ellen, 1978). Since remobilization of assimilated N requires degradation of leaf proteins and amino acids, it corresponds with leaf senescence and a decrease in photosynthetic activity. If soil N is limited or leaves are not producing enough photosynthate for both roots and developing grain, the plant is likely to slow or stop uptake after anthesis, which would make the N already present in the plant the only source of grain N at harvest. If more N remains in the leaves to maintain photosynthetic capacity, however, it is not available for remobilization to the grain, which could reduce grain protein concentration unless the plant can take up more soil N (Spano et al., 2003). Plants with rapid early-season N uptake, efficient remobilization and complete leaf senescence are likely to perform better in environments with severe N stress late in the season, but plants that are capable of late season uptake could have an advantage if environmental conditions make soil N available. Factors that contribute to late season uptake, such as increased root mass and depth, prolonged photosynthesis and greater leaf area can also help plants continue to fill grain when faced with drought stress.

2.6.2 Improved N Uptake Ability at Low Soil Concentrations

Some species and varieties of plants have overcome limited nutrient availability through symbiosis with arbuscular mycorrhizal (AM) fungi. Although this has received much attention for its role in phosphorus acquisition, the symbiosis may also be important in N uptake, either through direct N transfer or enhanced plant nutritional status leading to increased uptake capacity (Azcón et al., 2001). Mycorrhizal symbiosis contributed to enhanced nutrient use efficiency in an experiment with lettuce, especially at low N concentrations in the growth medium. At high concentrations, the proportion of N derived from fertilizer decreased, particularly in those plants with AM fungal colonization (Azcón et al., 2001). This implies that mycorrhizal colonization may be beneficial for those plants when fertilizer N is not readily available, but that the symbiosis does not necessarily aid in fertilizer N uptake. Plants with complementary root architecture explore and utilize different parts of the soil profile and may transfer nutrients through AM fungi (Lynch, 2004).

Unfortunately, the ability to form mycorrhizal symbiosis may have declined in wheat cultivars bred after the 1950s as a result of higher levels of soil fertility in breeding programs making such symbiosis unnecessary and even detrimental to plant growth. Hetrick et al. (1993) found that in wheat's wild ancestors, the roots of all

accessions tested were colonized by mycorrhizal symbionts but not all the accessions showed a positive growth response. For landraces, or traditional farmer varieties, mycorrhizal colonization did increase plant growth, with the strongest response observed in landrace accessions from Asia (China, Turkey, Afghanistan and Korea), leading the authors to speculate that perhaps the cultivation of wheat in monoculture without fertilization indirectly selected for increased mycorrhizal dependence (Hetrick et al., 1992). If landraces were selected for increased productivity compared to their wild ancestors, this could have increased both the demand for soil nutrients and plant nutrient losses because of the relationship between growth rate and nutrient turnover. Higher levels of root exudates could have favored the development of mycorrhizal symbiosis in early agricultural systems.

In more modern cultivars, the benefits plants received from colonization ranged from positive to neutral to negative. The fact that cultivars show variable responses to colonization suggests that there may be two sets of genes involved, one for the process of colonization and the other for nutrient acquisition by the plant (Hetrick et al., 1993). Winter wheat cultivars appeared to form beneficial symbiosis up until 1950, but cultivars released after that time have inconsistent relationships with mycorrhizae, including reduced growth after colonization. This may be a result of modern breeding in highly fertile soils, and it remains to be seen whether breeding programs deliberately conducting selection in low-input environments will select for increased colonization and symbiosis.

The effects of plant root exudates on the rhizosphere environment is of interest to low input and organic systems because of the potential to increase the availability of nutrients to the plant. Microbial biomass can strongly influence the dynamics of mineral N in the soil (Nieder et al., 1996). A full review of the soil-plant-microbial interactions affecting nutrient availability is beyond the scope of this paper, however, there is evidence that plant species and genotypes within species may have differential effects on rhizosphere microorganizms and nutrient availability (Rengel and Marschner, 2005; Cheng et al., 2003). With perennial crops, the ability of the plant to modify the rhizosphere environment may be even more critical. As plants release N-containing compounds through root sloughing when aboveground material senesces in autumn, having strong symbioses with mycorrhizae or beneficial root-zone microorganisms may enable the plant to recapture this N during spring regrowth, increasing the functional N_c. In a study of a native grass species, *Poa pratensis* L., which had coevolved with grazing animals, Hamilton and Frank (2001) found that defoliation of plants led to increased carbon exudates, which stimulated rhisospheric microbial population growth. This, in turn, led to higher levels of available N, which increased N uptake, shoot N and rates of photosynthesis in the recovering plants. Selection of plants with high levels of crown and tissue N plus useable rhizosphere N and vigorous spring growth may increase the performance of perennial grains more than selection only for grain yield and protein characteristics or for fast vegetative growth in autumn.

In addition to selecting for the ability to form symbioses, it is important to increase root affinity for soil N at low N concentrations. A study of wheat cultivars released over the past several decades shows that historic cultivars have better ability to extract and use soil N when there was no added fertilizer (Foulkes et al., 1998). In contrast, more recent cultivars were more responsive to high N supplies. It is likely that improvements in fertilizer use in modern varieties are due to increased rates of N uptake during the period following N fertilizer application. The decrease in soil N uptake capacity may be because of less vigorous early growth and rooting in semi-dwarf cultivars (Foulkes et al., 1998). A comparison of tall and semi-dwarf varieties showed that yields were similar under high-N conditions, but that tall genotypes performed better in low-N treatments (Morris and Paulsen, 1985). These changes over time indicate that modern breeding may have created varieties that are not well-suited to low-input or organic systems. These systems are much more complex than experimental systems where N is limiting and all other management factors are optimal. Because low-input and organic systems rely much more on biological cycling of nutrients, NUE in these systems will be closely linked to management and genetics that synchronize crop demands with N availability.

2.7 Conclusion

Low input and organic agricultural systems present unique environmental conditions and objectives not present in either conventional agriculture or natural ecosystems. The ideal crop plant would be adapted to low N conditions while still producing high yields of nutritious grain for human consumption. The evolutionary trade-off between high productivity and adaptation to low nutrient environments presents a challenge to this goal. However, plant breeders often select for increases in negatively correlated traits, and with careful selection strategies, it may be possible to improve NUE in both an agricultural and ecological context.

Breeding for high input systems may have increased the relative N requirement of crop plants, while natural selection on crop wild relatives has perhaps increased the mean residence time at the expense of N productivity. Because breeders are able to utilize germplasm from both elite cultivars and wild species, the beneficial traits from both of these gene pools may be combined and selected specifically for performance in sustainable agricultural systems. More research is needed to better understand the N dynamics of low input and organic systems and to develop effective selection tools. Methods of selecting both annual and perennial crops for symbiosis with soil microorganisms need to be developed. A better understanding of the partitioning of N in perennial species and the proportion of total N uptake that is carried over to the subsequent season is also needed.

Most likely, there is an optimal balance of N conservation and N productivity that falls somewhere between that of natural ecosystems and that of conventional agriculture. This optimum will depend on management practices and climactic conditions. To achieve both agricultural and ecological goals, it is necessary to develop crop varieties using a combination of agricultural and ecological NUE concepts.

Acknowledgments

The authors would like to thank two anonymous reviewers for their comments and suggestions. This material is based upon work supported by the National Science Foundation under a Graduate Research Fellowship and by The Land Institute. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation or The Land Institute.

Bibliography

Ackerly D.D., Dudley S.A., Sultan S.E., Schmitt J., Coleman J.S., Linder C.R., Sandquist D.R., Geber M.A., Evans A.S., Dawson T.E., Lechowicz M.J., 2000. The evolution of plant ecophysical traits: recent advances and future directions. BioScience 50(11), 979–995.

Aerts R., van der Peijl M.J., 1993. A simple model to explain the dominance of low-productive perennials in nutrient-poor habitats. Oikos 66(1), 144–147.

Agrama H.A.S., Zakaria A.G., Said F.B., Tuinstra M., 1999. Identification of quantitative trait loci for nitrogen use efficiency in maize. Molecular Breeding 5, 187–195.

Austin R.B., Ford M.A., Edrich J.A., Blackwell R.D., 1977. The nitrogen economy of winter wheat. Journal of Agricultural Science 88, 159–167.

Azcón, Ruiz-Lozano J.M., Rodriguez R., 2001. Differential contribution of arbuscular mycorrhizal fungi to plant nitrate uptake (^{15}N) under increasing N supply to soil. Canadian Journal of Botany 79, 1175–1180.

Badaruddin M., Meyer D.W., 1994. Grain legume effects on soil nitrogen, grain yield, and nitrogen nutrition of wheat. Crop Science 34, 1304–1309.

Baggs E.M., Watson C.A., Rees R.M., 2000. The fate of nitrogen from incorporated cover crop and green manure residues. Nutrient Cycling in Agroecosystems 56, 153–163.

Basra A.S., Goyal S.S., 2002. Mechanisms of improved nitrogen-use efficiency in cereals. In: Kang M.S. (ed.), Quantitative genetics, genomics and plant breeding, CABI publishing. pp. 269–288.

Berendse F., Aerts R., 1987. Nitrogen-use-efficiency: a biologically meaningful definition? Functional Ecology 1, 293–296.

Berry P.M., Sylvester-Bradley R., Philipps L., Hatch D.G., Cuttle S.P., Rayns F.W., Gosling P., 2002. Is the productivity of organic farms restricted by the supply of available N? Soil Use and Management 18(S1), 248–255.

Bertin P., Gallais A., 2000. Genetic variation for nitrogen use efficiency in a set of recombinant maize inbred lines I. Agrophysiological results. Maydica 45, 55–66.

Biederbeck V.O., Bouman O.T., Campbell C.A., Bailey L.D., Winkelman G.E., 1996. Nitrogen benefits from four green-manure legumes in dryland cropping systems. Canadian Journal of Plant Science 76, 307–315.

Borrell A.K., Hammer G.L., 2000. Nitrogen dynamics and the physiological basis of stay-green in sorghum. Crop Science 40, 1295–1307.

Bouldin D.R., Klausner S.D., Reid W.S., 1984. Use of nitrogen from manure. In: Hauck R.D. (ed.), Nitrogen in Crop Production, ASA-CSSA-SSSA. pp. 221–245.

Byers D., 2005. Evolution in heterogeneous environments and the potential of maintenance of genetic variation in traits of adaptive significance. Genetica 123, 107–124.

Campbell C.A., Zentner R.P., Selles F., Biederbeck V.O., Leyshon A.J., 1990. Comparative effects of grain lentil-wheat and monoculture wheat on crop production, N economy and N fertility in a Brown Chernozem. Canadian Journal of Plant Science 72, 1091–1107.

Cassman K.G., Dobermann A., Walters D.T., 2002. Agroecosystems, nitrogen-use efficiency and nitrogen management. Ambio 31(2), 132–140.

Ceccarelli S., 1996. Adaptation to low/high input cultivation. Euphytica 92, 203–214.

Chapin III F.S., 1980. The mineral nutrition of wild plants. Annual Review of Ecology and Systematics 11, 233–260.

Cheng W., Johnson D.W., Fu S., 2003. Rhizosphere effects on decomposition: Controls of plant species, phenology and fertilization. Soil Science Society of America Journal 67(5), 1418–1427.

Clarke J.M., Campbell C.A., Cutforth H.W., DePauw R.M., Winkleman G.E., 1990. Nitrogen and phosphorus uptake, translocation and utilization efficiency of wheat in relation to environment and cultivar yield and protein levels. Canadian Journal of Plant Science 70, 965–977.

Collins S.A., Allinson D.W., 2004. Soil nitrate concentrations used to predict nitrogen sufficiency in relation to yield in perennial grasslands. Agronomy Journal 96, 1272–1281.

Cook R.J., Sitton J.W., Haglund W.A., 1987. Influence of soil treatments on growth and yield of wheat and implications for control of pythium root rot. Phytopathology 77, 1192–1198.

Coque M., Gallais A., 2006. Genomic regions involved in response to grain yield

selection at high and low nitrogen fertilization in maize. Theoretical and Applied Genetics 112, 1205–1220.

Cox M., Qualset C.O., Rains D.W., 1985a. Genetic variation for nitrogen assimilation and translocation in wheat II: Nitrogen assimilation in relation to grain yield and protein. Crop Science 25, 435–440.

Cox M.C., Qualset C.O., Rains D.W., 1985b. Genetic variation for nitrogen assimilation and translocation in wheat I: Dry matter and nitrogen accumulation. Crop Science 25, 430–435.

Cox M.C., Qualset C.O., Rains D.W., 1986. Genetic variation for nitrogen assimilation and translocation in wheat III: Nitrogen translocation in relation to grain yield and protein. Crop Science 26, 737–740.

Dalal R.C., Strong W.M., Weston E.J., Cooper J.E., Wildermuth G.B., Lehane K.J., King A.J., Holmes C.J., 1998. Sustaining productivity of a Vertisol at Warra, Queensland, with fertilisers, no-tillage or legumes 5. wheat yields, nitrogen benefits and water-use efficiency of chickpea-wheat rotation. Australian Journal of Experimental Agriculture 38, 489–501.

Davis W.H., Middleton G.K., Hebert T.T., 1961. Inheritance of protein, texture and yield in wheat. Crop Science 1, 235–238.

Dhugga K.S., Waines J.G., 1989. Analysis of nitrogen accumulation and use in bread and durum wheat. Crop Science 29, 1232–1239.

Dou Z., Fox R.H., Toth J.D., 1994. Tillage effect on seasonal nitrogen availability in corn supplied with legume green manures. Plant and soil 162, 203–210.

Drinkwater L.E., Janke R.R., Rossoni-Longnecker L., 2000. Effects of tillage intensity on nitrogen dynamics and productivity in legume-based grain systems. Plant and Soil 227, 99–113.

Drinkwater L.E., Wagoner P., Sarrantonio M., 1998. legume-based cropping systems have reduced carbon and nitrogen losses. Nature 396, 262–265.

Dubois J.B., Fossati A., 1981. Influence of nitrogen uptake and nitrogen partitioning efficiency on grain yield and grain protein concentration of twelve winter wheat genotypes (*Triticum aestivum* L.). Zeitschrift fur Pflansensuchtung=Journal of Plant Breeding 86, 41–49.

Duncan R.R., Bockhold A.J., Miller F.R., 1981. Descriptive comparison of senescent and non-senescent sorghum genotypes. Agronomy Journal 73, 849–853.

Fiez T.E., Pan W.L., Miller B.C., 1995. Nitrogen efficiency analysis of winter wheat among landscape positions. Soil Science Society of America Journal 59, 1666–1671.

Foulkes M.J., Sylvester-Bradley R., Scott R.K., 1998. Evidence for differences between winter wheat cultivars in aquisition of soil mineral nitrogen and utilization of applied fertilizer nitrogen. Journal of Agricultural Science 130, 29–44.

Fowler D.B., Brydon J., Darroch B.A., Entz M.H., Johnston A.M., 1990.

Environment and genotype influence on grain protein concentration of wheat and rye. Agronomy Journal 82, 655–664.

Galloway J.N., Cowling E., Seltzinger S.P., Socolow R.H., 2002. Reactive nitrogen: too much of a good thing? Royal Swedish Academy of Sciences 31, 60–63.

Gauer L.E., Grant C.A., Gehl D.T., Bailey L.D., 1992. Effects of nitrogen fertilization on grain protein content, nitrogen uptake and nitrogen use efficiency of six spring wheat (*Triticum aestivum* L.) cultivars, in relation to estimated moisture supply. Canadian Journal of Plant Science 72, 235–241.

Górny A.G., 2001. Variation in utilization efficiency and tolerance to reduced water and nitrogen supply among wild and cultivated barleys. Euphytica 117, 59–66.

Halvorson A.D., Nielsen D.C., Reule C.A., 2004. Nitrogen fertilization and rotation effects on no-till dryland wheat production. Agronomy Journal 96, 1196–1201.

Hamilton E., Frank D.A., 2001. Can plants stimulate soil microbes and their own nutrient supply? Evidence from a grazing tolerant grass. Ecology 89(9), 2397–2402.

Harper L.A., Sharpe R.R., Langdale G.W., Giddens J.E., 1987. Nitrogen cycling in a wheat crop: Soil, plant and aerial nitrogen transport. Agronomy Journal 79, 965–973.

Hauggaard-Nielsen H., Ambus P., Jensen E.S., 2003. The comparison of nitrogen use and leaching in sole cropped versus intercropped pea and barley. Nutrient Cycling in Agroecosystems 65, 289–300.

Hetrick B.A.D., Wilson G.W.T., Cox T.S., 1992. Mycorrhizal dependence of modern wheat varieties, landraces and ancestors. Canadian Journal of Botany 70, 2032–2040.

Hetrick B.A.D., Wilson G.W.T., Cox T.S., 1993. Mycorrhizal dependence of modern wheat cultivars and ancestors: A synthesis. Canadian Journal of Botany 71, 512–518.

Hikosaka K., 2004. Interspecific difference in the photosynthesisnitrogen relationship: patterns, physiological causes, and ecological importance. J Plant Res 117, 481–494.

Huggins D.R., 1991. Redesigning no-tillage cropping systems: Alternatives for increasing productivity and nitrogen use efficiency. Ph.D. thesis, Washington State University.

Huggins D.R., 1993. Nitrogen efficiency component analysis: An evaluation of cropping system differences in productivity. Agronomy Journal 85(4), 898–905.

Huggins D.R., Pan W.L., 2003. Key indicators for assessing nitrogen use efficiency in cereal-based agroecosystems. Journal of Crop Production 8, 157–185.

Jiang Z., Hull R.J., 1998. Interrelationships of nitrate uptake, nitrate reductase, and nitrogen use efficiency in selected Kentucky Bluegrass cultivars. Crop Science 38, 1623–1632.

Jiang Z., Sullivan W.M., Hull R.J., 2000. Nitrate uptake and nitrogen use efficiency by Kentucky Bluegrass cultivars. Hort. Science 35(7), 1350–1354.

Johnson V., Schmidt J., Mattern P., 1968. Cereal breeding for better protein impact. Economic Botany 22, 16–25.

Johnson V.A., Mattern P.J., Schmidt J.W., 1967. Nitrogen relations during spring growth in varieties of *Triticum aestivum* L. differing in grain protein content. Crop Science 7, 664–667.

Kihlman-Falk E., 1961. Components of the uptake and transport of high accumulative ions in wheat. Physiologia Plantarum 14, 417.

Kramer A.W., Doane T.A., Horwath W.R., van Kessel C., 2002. Combining fertilizer and organic inputs to synchronize N supply in alternative cropping systems in california. Agriculture, Ecosystems and Environment 91, 233–243.

Lafitte H.R., Edmeades G.O., 1994. Improvement for tolerance to low soil nitrogen in tropical maize. I. Selection criteria. Field Crop Research 39, 1–14.

Lammerts van Bureren E., Struik P., Jacobsen E., 2002. Ecological concepts in organic farming and their consequences for an organic crop ideotype. NJAS - Wageningen Journal of Life Sciences 50, 1–26.

Li Y.S., Redmann R.E., van Kessel C., 1992. Nitrogen budget and ^{15}N translocation in a perennial wheat grass. Functional Ecology 6, 221–225.

Loffler C.M., Busch R.H., 1982. Selection for grain protein, grain yield and nitrogen partitioning efficiency in hard red spring wheat. Crop Science 22, 591–595.

Lynch J., 2004. Roots of the second green revolution. Paper presented at the ASA-CSSA-SSSA Annual Meetings, Seattle, WA: Oct. 31-Nov. 4.

Mahler R.L., Auld D.L., 1989. Evaluation of the green manure potential of Austrian winter peas in Northern Idaho. Agronomy Journal 81, 258–264.

Mason H.E., Spaner D., 2006. Competitive ability of wheat in conventional and organic management systems: a review of the literature. Canadian Journal of Plant Science 86, 333–343.

May L., Sanford D.A.V., MacKown C.T., Cornelius P.L., 1991. Genetic variation for nitrogen use in soft red x hard red winter wheat populations. Crop Science 31, 626–630.

McKendry A.L., McVetty P.B.E., Evans L.E., 1995. Selection criteria for combining high grain yield and high grain protein concentration in bread wheat. Crop Science 35, 1597–1602.

Moll R.H., Kamprath E.J., Jackson W.A., 1982. Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization. Agronomy Journal 74, 562–564.

Morris C.F., Paulsen G.M., 1985. Development of hard winter wheat after anthesis as affected by nitrogen nutrition. Crop Science 25, 1007–1010.

Murphy K.M., Campbell K.G., Lyon S.R., Jones S.S., 2007. Evidence of varietal adaptation to organic farming systems. Field Crops Research 102, 172–177.

Neales T.F., Anderson M.J., Wardlaw I.F., 1963. The role of the leaves in the accumulation of nitrogen by wheat during ear development. Australian Journal of Agricultural Research 14, 725–736.

Nieder M., Neugebauer E., Willenbockel A., Kersebaum K.C., Richter J., 1996. Nitrogen transformation in arable soils of North-West Germany during the cereal growing season. Biology and Fertility of Soils 22, 179–183.

Oritz-Monasterio J.I., Sayre K.D., Rajaram S., McMahon M., 1997. Genetic progress in wheat yield and nitrogen use efficiency under four nitrogen rates. Crop Science 37, 898–904.

Paccaud F.X., Fossati A., Cao H.S., 1985. Breeding for yield and quality in winter wheat: Consequences for nitrogen uptake and partitioning efficiency. Zeitschrift fur Pflanzenzuchtung = Journal of plant breeding 94(2), 89–100.

Pang X.P., Letey J., 2000. Organic farming: Challenge of timing nitrogen availability to crop nitrogen requirements. Soil Science Society of America Journal 64, 247–253.

Power J.F., Doran J.W., 1984. Nitrogen use in organic farming. In: Hauck R.D. (ed.), Nitrogen in Crop Production, ASA-CSSA-SSSA. pp. 585–598.

Presterl T., Groh S., Landbeck M., Seitz G., Geiger H.H., 2002. Nitrogen uptake and

utilization efficiency of European maize hybrids developed under conditions of low and high nitrogen input. Plant Breeding 121, 480–486.

Raun W.R., Johnson G.V., 1999. Improving nitrogen use efficiency for cereal production. Agronomy Journal 91, 357–363.

Redman M.H., Wigglesworth S.A., Vinten A.J.A., 1989. Nitrogen dynamics of a leguminous green manure. In: Hansen J.A., Henriksen K. (eds.), Nitrogen in Organic Wastes Applied to Soil, Academic Press. pp. 98–112.

Rengel Z., Marschner P., 2005. Nutrient availability and management in the rhizosphere: exploiting genotypic differences. New Phytologist 168, 305–312.

Sattlemacher B., Horst W.J., Becker H.C., 1994. Factors that contribute to genetic variation for nutrient efficiency of crop plants. Journal of Plant Nutrition and Soil Science (Zeitschrift fr Pflanzenernährung und Bodenkunde 157, 215–224.

Schoenau J.J., Campbell C.A., 1996. Impact of crop residues on nutrient availability in conservation tillage systems. Canadian Journal of Plant Science 76, 621–626.

Silla F., Escudero A., 2004. Nitrogen-use efficiency: trade-offs between N productivity and mean residence time at organ, plant and population levels. Functional Ecology 18, 511–521.

Singh V.P., Arora A., 2001. Intraspecific variation in nitrogen uptake and nitrogen utilization efficiency in wheat (*Triticum aestivum* L.). Journal of Agronomy and Crop Science 186, 239–244.

Soon Y., Clayton G., 2002. Effects of eight years of crop rotation and tillage on crop production and N fertilizer use. Canadian Journal of Soil Science 82, 165–172.

Soon Y., Clayton G., 2003. Effects of eight years of crop rotation and tillage on nitrogen availability and budget of a sandy loam soil. Canadian Journal of Soil Science 83, 475–481.

Soon Y.K., 1988. Root distribution of and water uptake by field-grown barley in a Black Solod. Can. J. Soil Sci. 68, 425–432.

Soon Y.K., Clayton G.W., Rice W.A., 2001. Tillage and previous crop effects on dynamics of nitrogen in a wheat-soil system. Agronomy Journal 93, 842–849.

Spano G., Fonzo N.D., Perrotta C., Platani C., Ronga G., Lawlor D.W., Napier J.A., Shewry P.R., 2003. Physiological characterization of stay green mutants in durum wheat. Journal of Experimental Botany 54(386), 1415–1420.

Spiertz J.H.J., Ellen J., 1978. Effects of nitrogen on crop development and grain growth of winter wheat in relation to assimilation and utilization of assimilates and nutrients. Neth. J. Agric. Sci. 26, 210–231.

Spillman W.J., Lang E., 1924. The Law of Diminishing Returns. World Book Company, New York.

Stockdale E.A., Shepherd M.A., Fortune S., Cuttle S.P., 2002. Soil fertility in organic farming systems - fundamentally different? Soil Use and Management 18, 301–308.

Stopes C., Millington S., Woodward L., 1996. Dry matter and nitrogen accumulation by three leguminous green manure species and the yield of a following wheat crop in an organic production system. Agriculture, Ecosystems and Environment 57, 189–196.

Strong W.M., Harbison J., Nielsen R.G.H., Hall B.D., Best E.K., 1986. Nitrogen availability in a Darling Downs soil following cereal, oilseed and grain legume crops. 2. Effects of residual soil nitrogen and fertilizer nitrogen on subsequent wheat crops. Australian Journal of Experimental Agriculture 26, 353–359.

Tilman D., 1986. Nitrogen-limited growth in plants from different successional stages. Ecology 67(2), 555–561.

Torstensson G., 1998. Nitrogen delivery and utilization by subsequent crops after incorporation of leys with different plant composition. Biological Agriculture and Horticulture 16, 129–143.

Tyson S.C., Cabrera M.L., 1993. Nitrogen mineralization in soils amended with composted and uncomposted poultry litter. Communications in Soil Science and Plant Analysis 24(17 and 18), 2361–2374.

Van Sanford D.A., MacKown C.T., 1986. Variation in nitrogen use efficiency among soft red winter wheat genotypes. Theoretical and Applied Genetics 72, 158–163.

Van Sanford D.A., MacKown C.T., 1987. Cultivar differences in nitrogen remobilization during grain fill in soft red winter wheat. Crop Sci. 27, 295–300.

Vance C.P., 2001. Symbiotic nitrogen fixation and phosphorus acquisition: Plant nutrition in a world of declining renewable resources. Plant Physiology 127, 390–397.

Vazquez de Aldana B., Berendse F., 1997. Nitrogen-use efficiency in six perennial grasses from contrasting habitats. Functional Ecology 11, 619–626.

Watson C.A., Atkinson D., Gosling P., Jackson L.R., Rayns F.W., 2002. Managing soil fertility in organic farming systems. Soil Use and Management 18(S1), 239–247.

Weston E.J., Dalal R.C., Strong W.M., Lehane K.J., Cooper J.E., King A.J., Holmes C.J., 2002. Sustaining productivity of a Vertisol at Warra, Queensland, with fertilisers, no-tillage or legumes 6. production and nitrogen benefits from annual medic in rotation with wheat. Australian Journal of Experimental Agriculture 42, 961–969.

Wilkins P.W., Allen D.K., Mytton L.R., 1999. Differences in the nitrogen use efficiency of perennial ryegrass varieties under simulated rotational grazing and their effects on nitrogen recovery and herbage nitrogen content. Grass and Forage Science 55, 69–76.

Woodruff D., 1972. Cultivar variation in nitrogen uptake and distribution in wheat. Australian Journal of Experimental Agriculture and Animal Husbandry 12, 511–516.

Zemenchik R., Albrecht K.A., 2002. Nitrogen use efficiency and apparent nitrogen recovery of Kentucky Bluegrass, Smooth Bromegrass and Orchardgrass. Agronomy Journal 94, 421–428.

Chapter 3

Comparison of winter wheat genotypes selected under different nitrogen regimes for traits related to nitrogen use in an organic system

Abstract

Organic and conventional agricultural systems differ significantly in terms of soil nitrogen(N) cycling and crop nitrogen use efficiency (NUE). To breed crops with improved NUE in organic systems, breeders must determine whether there is genetic variation for traits related to NUE and identify sources of traits related to higher NUE. The goals of this study were to compare variation in yield, aboveground biomass (AGB) and grain protein traits among winter wheat breeding lines selected in conventional and organic systems, historic wheat varieties and a perennial wheat population grown in an organic system. There was significant genetic variation in

this sample of 22 genotypes for grain yield, grain %N, grain total N (TN) and AGB. The breeding category (conventional, organic, historic or perennial) also had a significant effect, as only three of 24 contrasts among categories were non-significant. Environmental effects and interactions between genotype and environment were also significant for all four response variables. Regression analysis showed a negative relationship between grain %N and grain yield, and no genotypes were identified with significant grain protein deviations (GPD) (large standardized residuals from this regression). These results indicate that there is significant genetic variation present for traits related to NUE in an organic system and that selection in the target environment may be the most effective way to improve this complex trait.

Keywords: winter wheat, nitrogen use efficiency, organic management, selection, specific adaptation

3.1 Introduction

Breeding crop varieties specifically for organic and low-input systems is important due to the unique constraints and challenges of these systems, particularly related to nitrogen availability. Varieties selected under conventional agricultural management practices may not have optimal traits for organically managed systems. The top yielding wheat genotypes in conventional systems were not the same as the top yielding genotypes in organic systems in four out of five site-years when tested

side-by-side at locations in Eastern Washington (Murphy et al., 2007). This evidence of adaptation to a specific agricultural system suggests that selection in organic systems could have significant benefits and may be just as important as selection in the target geographical region.

Organic and low-input systems depend on N mineralization from organic forms and biological N cycling. In dryland organic systems such as are found in Eastern Washington, the pool of available N may be small during much of the year but there may be flushes of mineralization due to moisture and temperature fluctuations. Consequently, prediction of available N supplies is more difficult than in agricultural systems that rely on the application of synthetic fertilizers. Breeding crops under an N regime that is managed to limit any deficiencies may have created varieties which are dependent on readily available N relatively throughout the growing season for optimal performance (Foulkes et al., 1998). These varieties may not be able to use N efficiently in organic systems where N cycling and flow create more heterogeneous supplies of available N. Breeding strategies that simply utilize a nursery without inorganic fertilizer N would not capture the range of stresses or the biological interactions present in organic and low-input systems. Watson et al. (2002) states that research trials without added fertilizer N are not an accurate representation of organic systems.

Even though the importance of nitrogen use efficiency (NUE) is widely recognized and studied, there are many conflicting results due to different

germplasm, environmental and experimental conditions, and definitions of NUE itself (Huggins and Pan, 2003; Van Sanford and MacKown, 1987; Fowler et al., 1990). NUE is an inherently complex trait because it is a function of interacting genetic and environmental factors, such as the amount and distribution of precipitation over the growing season, mean temperatures and variation, soil quality including structure and microbial activity, cropping history and biotic constraints such as weeds, insects and pathogens. The traditional definition of NUE as grain weight/ N supply (Moll et al., 1982) often leads researchers to focus on maximizing the yield response to fertilizer N rather than maintaining or increasing yield levels with lower levels of N. In breeding for organic and low input systems, selection must emphasize the effective use of organic and indigenous supplies of available N while increasing yields and meeting grain quality targets.

Because most modern wheat varieties have been selected under synthetic N management, beneficial traits for NUE in low-input and organic systems may have been lost. In a study of wheat varieties released over several decades, older wheat varieties had a better ability to extract and use soil N when there was no added fertilizer, while more recent cultivars were more responsive to high N supplies (Foulkes et al., 1998). This may be due to less vigorous early growth in semi-dwarf cultivars. A comparison of semi-dwarf and standard height varieties showed that there was no difference in yield under high-N conditions, but standard height varieties yielded more in low-N conditions (Morris and Paulsen, 1985). However,

while historic varieties developed under conditions of low N input may have important traits for low-input and organic agriculture, it is also possible that modern varieties have traits important for N-uptake because increases in the harvest index (HI) would require the plant to assimilate more N for the same amount of aboveground biomass (AGB) since grain has a higher N content than stover and chaff. Breeders may have been indirectly selecting for improved N uptake while increasing yield and HI (Sinclair, 1998).

In addition to historic annual wheat varieties, wild relatives of wheat may have traits important to N uptake in organic systems. In perennial wheatgrass, natural selection has been acting on species in competitive prairie ecosystems where N is limited. Deep root systems and longer photosynthetic duration may indicate that perennials are more efficient at capturing and using N. In a study of wild and domesticated perennial grasses, Chapin (1980) and Jiang and Hull (1998) found that species adapted to low N conditions were able to take up more N at low concentrations than those adapted to high N conditions and vice-versa. The winter wheat breeding program at Washington State University (WSU WW) has been developing perennial wheat through crossing annual winter wheat *Triticum aestivum* L. with *Thinopyrum* spp. and backcrossing to modern varieties of annual winter wheat. This has successfully created perennial wheat that is phenotypically similar to winter wheat, and which has been selected for regrowth in subsequent years. A bulk population of this material was included in the study for comparison with

annual wheat.

If sufficient genetic variation exists for NUE measured in organic and low input systems, it will be possible to make progress in breeding for improved NUE through selection for improved yields and adequate protein under organic N management. Studies on NUE in conventional systems have established that significant genetic variation exists for traits related to NUE, reviewed in chapter 2. However, genetic effects may be specific to the location and system because the genotype by environment (GxE) interaction often has a larger effect than the genotypic contribution itself (Bertin and Gallais, 2000). To establish and quantify the genetic and environmental effects on traits related to NUE in low-input systems, this study examined a diverse range of winter wheat germplasm under organic conditions in Eastern Washington. Our hypothesis was that lines selected in low input and organic systems, specifically the organically bred genotypes, historic varieties and perennial bulk population, would potentially have better ability to take up and use N from organic sources.

3.2 Materials and Methods

3.2.1 Germplasm and management

The experiment was conducted from 2005-2007 at two locations, with plantings in October of 2005 and 2006. The sites are transitional organic ground at Spillman Agronomy Farm (Spillman) in Pullman, WA (46 ° 73' N, 117 ° 18' W), and Sara and Joe DeLongs' (the Delong's) certified organic farm in St. John, WA (46 ° 59' N, 117 ° 33' W). Both sites are in Whitman County, in the Palouse region of Eastern Washington. At Spillman, the experimental plots are located on a Palouse Silt Loam soil classification and the field is in its second year of transition to being certified organic. At the DeLongs' farm, the fields are certified organic, on a Snow Silt Loam soil classification in 2005-2006 and on a Mondovi Silt Loam soil classification in 2006-2007. Pullman receives an annual average of 540 mm precipitation, and St. John receives 428 mm. Most precipitation occurs as rain or snow during the fall and winter months, and summers are generally hot and dry (Western Regional Climate Center 2005; www.wrcc.dri.edu).

Table 3.1 lists the plant materials used. Each annual genotype and the perennial population were planted in 3.5 m^2 plots in mid-October using a randomized complete block design (RCBD) at two locations with four replicates each. Perennial plots were planted each year and only evaluated the first year of growth. The annual wheat types include organically bred F_5 lines, conventionally bred F_5 lines and

historic varieties. All annual types are soft white winter wheat, except historic varieties which are cold-hardy soft white spring wheat, and White Marquis, which was reclassified as a hard white wheat during the study. The perennial bulk population was developed in the WSU WW. Organically bred lines have been grown and selected from the first crosses under USDA certified organic management practices (United States Department of Agriculture, 2000) developed by the WSU WW and certified by the Washington State Department of Agriculture. Conventionally bred lines have been grown and selected using the standard management practices of the WSU WW, including synthetic fertilizers. Historic varieties were chosen from screening trials conducted by the WSU WW of varieties grown in the Pacific Northwest prior to 1940, before the use of synthetic fertilizers in breeding programs and on farms became common in the 1950's. The annual genotypes were selected based on performance data from the 2005 organic, conventional and historic wheat trials, with six lines chosen for each category based on high respective grain yields and disease resistances. Check plots of Madsen (Allan et al., 1989) and J99C0009 were included as entries. Madsen is a popular soft white winter wheat in this region and one parent of all the organically bred genotypes. J99C0009 is a Madsen derivative with foot rot (*Pseudocercosporella herpotrichoides*) resistance and is one parent of all the conventionally bred genotypes.

The experimental plots followed peas *Pisum sativum* L. plowed under as a green manure each year at Spillman and followed fallow the first year and a

Table 3.1: Genotypes, pedigrees and classification of historic wheat varieties and breeding lines from WSU used in the study

Genotype	Pedigree	Category
Bulk	Madsen//Chinese Spring/*Thinopyrum spp.*	Perennial
Bunyip	Rymer/Maffra	Historic (released 1901)
Hyper	Pacific Bluestem/Prelude	Historic (released 1929)
Idaed	Sunset/Boadicea	Historic (released 1938)
Onas	Federation/Tarragon	Historic (released 1915)
Sonora	Collected Durango, Mexico	Historic (introduced 1907)
White Marquis	Mutant in Marquis	Historic [1]
5K020007	Onas/Madsen	Organic selection
5K020023	Madsen/Surprise	Organic selection
5K020082	Idaed/Madsen	Organic selection
5K020095	White Marquis/Madsen	Organic selection
5K020106	Currawa/Madsen	Organic selection
5K020138	Pacific Bluestem/Madsen	Organic selection
4J020274	Lewjain/J99C0009	Conventional selection
4J020275	Lewjain/J99C0009	Conventional selection
4J020185	Albion/J99C0009	Conventional selection
4J020187	Albion/J99C0009	Conventional selection
4J020259	J99C0009/Sorbas	Conventional selection
4J020210	Eltan/J99C0009	Conventional selection
Madson	VPM1/Moisson951//2*Hill81	Check
J99C0009	Spitzer/Madson	Check
[1]Marquis pedigree: Hard Red Calcutta/Red Fife, released 1910		
Pedigree information for conventional, organic and perennial lines: WSU WW Pedigree information for historic varieties from National Genetic Resources Program. Germplasm Resources Information Network (GRIN). [Online Database] National Germplasm Resources Laboratory, Beltsville, Maryland. Available: http://www.ars-grin.gov/cgi-bin/npgs/acc/display.pl?1650984 (01 Jan. 2007)		

harvested pea crop the second year at the DeLongs' farm. Joe DeLong used a manure spreader to apply hog manure and bedding during the fallow preceding the wheat crop planted in 2005. Due to a drought in late 2004 and early 2005, the spring pea green manure at Spillman only provided about 35 kg mineralizeable N per hectare to the crop planted in the fall of 2005. In addition, each experimental plot at Spillman was fertilized with Perfect Blend 4-4-4 NPK enhanced organic fertilizer (granulated poultry manure) at a rate of 42 kg N per hectare in early spring. No additional fertilizer was applied at the DeLongs' farm due to higher soil test N in that location. The breeding program does not use pesticides to control diseases or insect pests because of the desire to select for resistant genotypes. The DeLongs use mechanical methods of weed control, such as harrowing, and no other pest control methods. Weeds in this study were controlled with mechanical methods and limited hand weeding to reduce variation in weed pressure among the experimental plots.

3.2.2 Soil and plant sampling

Soil samples were taken twice each year, before planting and in the spring. Two cores were taken in each block. Each core went down to six feet and each foot was analyzed for inorganic N and soil moisture. Soil moisture content was determined on half of each sample by the gravimetric method. Samples were weighed immediately upon returning from the field, oven dried at 105 C for 24 hours and reweighed to determine % moisture. The other half of the sample was packed in ice and frozen to

prevent volatilization loss before analysis for inorganic N. Nitrate (NO_3^-) and ammonium N (NH_4^+) was extracted using KCL (Keeney and Nelson, 1982) and analyzed using a flow injection analyzer (FIA)(Lachat Instruments, Loveland, CO).

During the growing season, early stand vigor was determined visually using a scale of 1-5 in March each year. Leaf chlorophyll content was measured using a SPAD chlorophyll meter (Minolta Co, Japan). SPAD readings have been used to predict N uptake and grain yield in cereal grains and forage grasses (Vidal et al., 1999; Gáborčík, 2003; Giunta et al., 2002). Readings were taken three times during the growing season, corresponding to the feeke' scale of wheat growth stages 7 (SPAD1), 10.5-10.5.1 (SPAD2), and 10.5.3-10.5.4 (SPAD3). For each reading, five plants were chosen at random in each plot and four readings were taken along the youngest fully expanded leaf. Readings from the plots were averaged for the analysis.

Plant samples from each plot were taken at maturity. A 60 cm long segment of a row from the plot was selected at random and plants were cut at ground level. The bundles were weighed, then threshed and the grain was weighed. Harvest index (HI) was calculated as grain weight/total weight. Plots were combined with a Wintersteiger Nurserymaster (Wintersteiger Division of Seed Machinery, Salt Lake City, Utah), and grain was weighed to determine plot yield. A sample of grain was analyzed for protein content on a 12% moisture basis by near infrared (NIR) measurement using a Tecator Infratec 1226 Grain Analyzer (Foss Inc., Eden Prairie, MN). Plots that did not produce enough grain for the NIR were analyzed with a

CNS-2000 Elemental Analyzer (LECO corporation, St. Joseph, MI) for grain %N. Samples from more productive plots of the same varieties and the check genotypes were used to calibrate the measurements with those from the NIR. Grain protein was converted to %N by dividing by 5.74. Grain total N (TN) was calculated by multiplying grain yield by %N.

3.2.3 Analysis

ANCOVA models in SAS (SAS Institute, Cary, NC) were used to compare variation among and within the wheat types for grain yield, grain %N, grain TN and AGB production. Separate models were used to analyze genotypic effects (nested within category) and the effects of selection category (conventional, organic, historic and perennial). SPAD meter readings were used as quantitative covariates in the model to test for significant correlations between SPAD readings and the dependent variables. Only SPAD3 was significant for yield and grain TN, so this was the only covariate maintained in the final model. For grain %N, SPAD2 was also significant so the model included both SPAD2 and SPAD3. None of the SPAD meter readings were significant for AGB production, so this model became an ANOVA with no covariates. Because of increasing variance (larger residuals) as AGB production increased, a logarithmic transformation was used to stabilize the variance for the AGB ANOVA. Least squares (LS) mean values were calculated for each category and genotype for each dependent variable and Tukey's method was used for all pairwise comparisons.

A series of orthogonal contrasts was used to compare selection categories.

A regression analysis was also used to examine the relationship between grain %N, grain TN and yield. In particular, it was considered important to identify genotypes that had a higher or lower grain protein concentration (measured by %N) than would be expected for their yield. The methods of Oury and Godin (2007) were used to determine the standardized residuals, or grain protein deviation (GPD), from a regression of grain %N on grain yield. Regressions of grain TN on grain %N and grain TN on yield were also performed to explore the relationship between these three variables and the relative merits of selecting on grain TN and GPD.

3.3 Results

3.3.1 Effects of selection category, genotype and environmental factors

The selection category was significant at the $p < 0.05$ level only for grain percent N. Genotypic effects were significant for all the dependent variables (Table 3.2). Year and location also had significant effects on grain %N in both models but not on grain yield, grain TN or AGB production. SPAD2 and SPAD3 slopes were positive and small but significant for grain %N and grain TN. SPAD 3 slopes for yield were more substantial. Interaction effects between category and year

and between genotype and year were significant for all four dependent variables, however, interactions between location and category ($p = 0.066$) and location and genotype ($p = 0.0063$) were only significant for grain %N. The three way interaction year by location by genotype was significant for grain yield ($p = 0.0153$) and AGB ($p = 0.0045$). In addition, the block effect was always significant, probably due to high weed pressure which could not be completely standardized. In natural ecosystems such as prairies, plants must be extremely competitive in order to obtain enough N for their needs. Crop competition with weeds for available N is one potential trait of interest in organic systems, so hand weeding was not used to eliminate all weed pressure. The blocks were most significant at St. John in 2007, and less often at Spillman, where the weed pressure was less severe and more uniform. Blocks were less significant in 2006 when the weed infestation at both sites was less. The presence of a strong block effect demonstrates the heterogeneity of experimental conditions and the need for replication over space and time. Because the block effect can be statistically accounted for, it is still possible to draw conclusions about genetic and environmental effects.

Comparing the means of different categories gave information on the relative performance of each selection category and the perennial bulk population. For grain yield, AGB and grain TN, the check genotypes Madsen and J99C099 had the highest yield, but this was not significantly different from the conventional genotypes. The organic, historic and perennial categories had significantly lower yield, AGB

Table 3.2: Significance values for field study ANCOVA F-tests. Separate ANCOVA models were used for grain %N, grain TN, and grain yield. An ANOVA with a logarithmic transformation to stabilize the variance was used for AGB

Category model	Dependent variable			
Factor	AGB	Grain %N	Grain TN	Grain yield
SPAD2	-	0.0033	-	-
SPAD3	-	0.0004	0.0095	0.0095
block	0.0025	0.0002	0.0001	<.0001
year	0.4143	0.0049	0.9320	0.5271
location	0.5662	0.0168	0.4926	0.7972
category	0.1432	0.0223	0.0542	0.0683
year*category	0.0395	0.0077	0.0340	0.0046
loc*category	0.1327	0.0665	0.1184	0.1238
loc*year	0.0272	0.2476	0.0160	0.0006
year*loc*cat	0.1313	0.8500	0.3303	0.6547
SPAD 3 slope	-	0.006313	0.6312	31.8700
SPAD 2 slope	-	0.006699	-	-
Genotype model	Dependent variable			
Factor	AGB	Grain %N	Grain TN	Grain yield
SPAD2	-	<.0001	-	-
SPAD3	-	<.0001	0.0017	0.0005
block	0.0003	<.0001	<.0001	<.0001
year	0.3434	0.0002	0.8532	0.5044
location	0.4958	0.0213	0.6016	0.9323
genotype	0.0114	<.0001	0.0022	0.0025
year*genotype	0.0454	0.0003	0.0431	0.0072
loc*genotype	0.6083	0.0063	0.5312	0.7828
loc*year	0.0014	0.1894	0.0067	0.0010
year*loc*gen	0.0045	0.8444	0.0776	0.0153
SPAD 3 slope	-	0.007195	0.9151	48.5619
SPAD 2 slope	-	0.008767	-	-

Table 3.3: Comparison of selection categories for aboveground biomass, grain yield, grain %N, and grain TN using orthagonal contrasts

	Dependent variable			
Contrast	AGB	Grain yield	Grain %N	Grain TN
conventional vs. historic	***	***	***	***
conventional vs. organic	***	***	**	***
conventional vs. perennial	***	***	***	***
organic vs. historic	***	***	***	***
organic vs. perennial	ns	***	***	***
historic vs. perennial	***	ns	***	ns
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns - not significant				

production and grain TN than both the conventional and check genotypes. The perennial bulk population had the highest grain %N and all other categories, including the check genotypes, had significantly lower grain %N.

Only three of 24 contrasts were non-significant, showing that most of the selection categories were significantly different from each other in terms of yield, grain %N, grain TN and AGB production. The exceptions to this were that organic was not significantly different from perennial for AGB production, and historic was not significantly different from perennial for grain TN or grain yield (Table 3.3). Comparing the LS mean values of selection categories showed a definite pattern, with the check and conventional being higher for grain yield, grain TN and AGB followed by organic, historic and perennial for grain yield and grain TN, and followed by organic, perennial and historic for AGB. For grain %N, the ranking was almost exactly reversed, with perennial followed by historic, organic, conventional and check genotypes (Table 3.4).

Table 3.4: LS mean values and 95% confidence limits (in parentheses) for individual selection categories of wheat for the measured variables aboveground biomass, grain yield, grain %N, and grain TN

Category	AGB (kg/ha)*	Grain yield (kg/ha)	Grain %N	Grain TN (kg/ha)
check	13 200 (11 700 - 14 900)	5 053 (4 758 - 5 348)	1.71 (1.67 - 1.74)	85.7 (80.0 - 91.4)
conventional	12 800 (11 700 - 13 900)	4 762 (4 554 - 4 969)	1.75 (1.72 - 1.77)	81.6 (77.6 - 85.6)
organic	9 800 (9 000 - 10 700)	4 158 (3 953 - 4 362)	1.78 (1.76 - 1.81)	69.0 (65.0 - 72.9)
historic	6 240 (5 800 - 6 800)	2 789 (2 594 - 2 985)	1.91 (1.89 - 1.94)	47.1 (43.3 - 50.9)
perennial	8 830 (7 700 - 10 200)	2 518 (2 159 - 2 876)	2.05 (2.00 - 2.09)	46.8 (40.0 - 53.7)

3.3.2 Comparison of genotype LS mean values

The ranking for genotypes generally followed the category means ranking (Table 3.5, Table 3.6, Table 3.7, Table 3.8), however, genotypes with significantly greater or lower yield, %N, grain TN and AGB than other genotypes in their category were identified. In particular, organic genotype 5K020007 had high grain TN, not significantly different from the conventional genotypes and significantly greater than all the historic genotypes and organic genotypes 5K020138 and 5K020106. Its yield is also greater than all the historic genotypes and 5K020106. It has significantly lower protein than the perennial bulk, but significantly higher protein than Madsen, one of its parents, and two other conventional and organic breeding lines. Conventional genotypes 4J020275 and 4J020210-6 both out-yielded the conventional genotype 4J020259, three of the organic genotypes and all the historic genotypes. For grain TN, 4J020210-6 and 4J020274 had higher means than two organic genotypes and all the historic varieties. In terms of grain %N, 4J020275 was lower than all but four other genotypes, but 4J020274 and 4J020210-6 were only

significantly lower than four and eight other genotypes, respectively.

3.3.3 Relationship between grain N and grain yield

Grain yield and grain %N were negatively correlated (Table 3.9). Grain %N was also negatively correlated to grain TN and grain yield showed a strong positive correlation to grain TN. From the regression of grain %N on grain yield, no genotypes were identified with standardized residuals greater than two standard deviations (+/-1.96), the criteria for significant GPD from Oury and Godin (2007). Regressing grain %N on grain TN had an R^2 of 0.1098 and a slope of -0.0083, confirming that these two variables are not strongly correlated. This type of analysis could be useful for experiments with a wider range of grain %N, but for this study there were no significant deviations from the regression line.

3.4 Discussion

3.4.1 Sources of variation

It is apparent that there is significant genetic variation for traits related to N use in an organic system. The breeding history of these genotypes, especially their pedigrees, probably had a large impact on their performance. The organically bred genotypes might have had lower yield, grain TN and AGB than the conventionally

Table 3.5: Genotype LS mean values, 95% confidence intervals (Upper and Lower Confidence Limits for individual means) and pairwise comparisons for grain yield. Overall error rate controlled by Tukey's procedure for multiple comparisons

Genotype	J99C0009	4J020275	4J020210-6	4J020274	4J020187	5K020007	Madsen1	Madsen2	5K020095	5K020023	4J020185	4J020259	5K020082	5K020138	5K020106	WhiteMarq2	WhiteMarq1	Sonora	Idaed	Bunyip	Bulk1	Bulk2	Onas	Hyper
LSMean	5303	5140	5110	4946	4931	4878	4873	4849	4613	4519	4355	3931	3853	3723	3385	3356	3235	2924	2688	2665	2599	2490	2391	2384
LCL	4859	4703	4664	4510	4494	4409	4433	4392	4159	4060	3872	3495	3416	3286	2948	2902	2776	2457	2200	2199	2144	2050	1955	1947
UCL	5747	5577	5556	5383	5367	5346	5313	5306	5068	4978	4839	4367	4289	4160	3823	3810	3695	3390	3177	3130	3055	2929	2827	2821
entry	**18**	**6**	**3**	**5**	**2**	**7**	**19**	**20**	**10**	**8**	**1**	**4**	**9**	**12**	**11**	**24**	**23**	**22**	**17**	**15**	**13**	**14**	**21**	**16**
18	.	1	1	1	1	0.9999	0.9996	0.9993	0.8901	0.7097	0.3655	0.0047	0.0018	0.0002	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
6	1	.	1	1	1	1	1	1	0.9941	0.9596	0.7597	0.0275	0.0115	0.0022	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
3	1	1	.	1	1	1	1	1	0.9975	0.9757	0.8029	0.044	0.0203	0.0035	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
5	1	1	1	.	1	1	1	1	1	0.9997	0.9823	0.1737	0.0897	0.0232	0.0003	0.0003	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
2	1	1	1	1	.	1	1	1	1	0.9999	0.9874	0.1977	0.1042	0.0281	0.0004	0.0004	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
7	0.9999	1	1	1	1	.	1	1	1	1	0.9988	0.3499	0.1973	0.0835	0.0022	0.0014	0.0002	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
19	0.9996	1	1	1	1	1	.	1	1	1	0.9965	0.3079	0.1785	0.0515	0.0008	0.001	0.0002	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
20	0.9993	1	1	1	1	1	1	.	1	1	0.9987	0.3999	0.2479	0.0815	0.0017	0.0019	0.0005	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
10	0.8901	0.9941	0.9975	1	1	1	1	1	.	1	1	0.898	0.7663	0.4542	0.0288	0.0285	0.0087	0.0002	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
8	0.7097	0.9596	0.9757	0.9997	0.9999	1	1	1	1	.	1	0.979	0.9247	0.6881	0.0753	0.0774	0.0287	0.0009	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
1	0.3655	0.7597	0.8029	0.9823	0.9874	0.9988	0.9965	0.9987	1	1	.	0.9999	0.9983	0.9628	0.3299	0.3324	0.1707	0.0117	0.0008	0.0006	0.0001	<.0001	<.0001	<.0001
4	0.0047	0.0275	0.044	0.1737	0.1977	0.3499	0.3079	0.3999	0.898	0.979	0.9999	.	1	1	0.9883	0.9822	0.8823	0.2337	0.0407	0.022	0.009	0.0016	0.0004	0.0004
9	0.0018	0.0115	0.0203	0.0897	0.1042	0.1973	0.1785	0.2479	0.7663	0.9247	0.9983	1	.	1	0.9986	0.9973	0.9616	0.3803	0.0845	0.0476	0.0219	0.0044	0.0012	0.0012
12	0.0002	0.0022	0.0035	0.0232	0.0281	0.0835	0.0515	0.0815	0.4542	0.6881	0.9628	1	1	.	1	1	0.9983	0.7157	0.2398	0.1735	0.0858	0.0233	0.0064	0.0058
11	<.0001	<.0001	<.0001	0.0003	0.0004	0.0022	0.0008	0.0017	0.0288	0.0753	0.3299	0.9883	0.9986	1	.	1	1	0.9994	0.9144	0.866	0.7163	0.4196	0.2071	0.1916
24	<.0001	<.0001	<.0001	0.0003	0.0004	0.0014	0.001	0.0019	0.0285	0.0774	0.3324	0.9822	0.9973	1	1	.	1	0.9998	0.951	0.9098	0.7983	0.5136	0.2914	0.2829
23	<.0001	<.0001	<.0001	<.0001	<.0001	0.0002	0.0002	0.0005	0.0087	0.0287	0.1707	0.8823	0.9616	0.9983	1	1	.	1	0.9957	0.9874	0.9573	0.7915	0.5782	0.5772
22	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0002	0.0009	0.0117	0.2337	0.3803	0.7157	0.9994	0.9998	1	.	1	1	1	0.9997	0.9944	0.994
17	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0008	0.0407	0.0845	0.2398	0.9144	0.951	0.9957	1	.	1	1	1	1	1
15	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0006	0.022	0.0476	0.1735	0.866	0.9098	0.9874	1	1	.	1	1	1	1
13	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0001	0.009	0.0219	0.0858	0.7163	0.7983	0.9573	1	1	1	.	1	1	1
14	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0016	0.0044	0.0233	0.4196	0.5136	0.7915	0.9997	1	1	1	.	1	1
21	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0004	0.0012	0.0064	0.2071	0.2914	0.5782	0.9944	1	1	1	1	.	1
16	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0004	0.0012	0.0058	0.1916	0.2829	0.5772	0.994	1	1	1	1	1	.

Table 3.6: Genotype LS mean values, 95% confidence intervals (Upper and Lower Confidence Limits for individual means) and pairwise comparisons for grain %N. Overall error rate controlled by Tukey's procedure for multiple comparisons

Genotype	Bulk1	Bulk2	WhiteMarq1	Hyper	Bunyip	WhiteMarq2	Idaed	5K020082	Sonora	5K020007	4J020259	4J020274	4J020185	5K020106	5K020095	4J020210-6	Onas	J99C0009	5K020138	4J020187	Madsen1	Madsen2	5K020023	4J020275
LSMean	2.10	2.02	2.00	1.97	1.94	1.94	1.92	1.91	1.90	1.88	1.81	1.79	1.79	1.78	1.77	1.77	1.77	1.76	1.72	1.71	1.70	1.65	1.64	1.58
LCL	2.05	1.97	1.95	1.92	1.89	1.89	1.87	1.86	1.84	1.82	1.76	1.74	1.74	1.73	1.72	1.72	1.72	1.71	1.67	1.66	1.65	1.60	1.58	1.53
UCL	2.15	2.07	2.05	2.02	1.99	1.99	1.97	1.96	1.95	1.93	1.86	1.84	1.85	1.83	1.82	1.82	1.82	1.81	1.77	1.76	1.75	1.70	1.69	1.63
Entry	**13**	**14**	**23**	**16**	**15**	**24**	**17**	**9**	**22**	**7**	**4**	**5**	**1**	**11**	**10**	**3**	**21**	**18**	**12**	**2**	**19**	**20**	**8**	**6**
13	_	0.839	0.4945	0.0848	0.0055	0.006	0.0003	0.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
14	0.839	_	1	0.9999	0.8925	0.8946	0.4944	0.3307	0.1467	0.0309	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
23	0.4945	1	_	1	0.9941	0.9939	0.8646	0.7273	0.433	0.1302	0.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
16	0.0848	0.9999	1	_	1	1	0.9989	0.9929	0.9496	0.709	0.0023	0.0003	0.0006	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
15	0.0055	0.8925	0.9941	1	_	1	1	1	1	0.9916	0.0919	0.0204	0.0502	0.0062	0.0027	0.0038	0.0012	0.0014	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
24	0.006	0.8946	0.9939	1	1	_	1	1	1	0.994	0.1082	0.0199	0.0485	0.0081	0.0026	0.0041	0.0013	0.0015	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
17	0.0003	0.4944	0.8646	0.9989	1	1	_	1	1	1	0.2702	0.0782	0.1203	0.019	0.0115	0.0101	0.0068	0.0041	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
9	0.0001	0.3307	0.7273	0.9929	1	1	1	_	1	1	0.4159	0.1423	0.2104	0.0417	0.025	0.0235	0.0149	0.0102	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
22	<.0001	0.1467	0.433	0.9496	1	1	1	1	_	1	0.8	0.447	0.5908	0.228	0.1407	0.1603	0.0874	0.0862	0.0014	0.0002	<.0001	<.0001	<.0001	<.0001
7	<.0001	0.0309	0.1302	0.709	0.9916	0.994	1	1	1	_	0.9901	0.8825	0.9405	0.6763	0.5369	0.576	0.3968	0.4097	0.0197	0.0034	0.0015	<.0001	<.0001	<.0001
4	<.0001	<.0001	0.0001	0.0023	0.0919	0.1082	0.2702	0.4159	0.8	0.9901	_	1	1	1	1	1	1	0.9996	0.6768	0.3444	0.1774	0.0027	0.0007	<.0001
5	<.0001	<.0001	<.0001	0.0003	0.0204	0.0199	0.0782	0.1423	0.447	0.8825	1	_	1	1	1	1	1	1	0.9453	0.7151	0.4819	0.0166	0.005	<.0001
1	<.0001	<.0001	<.0001	0.0006	0.0502	0.0485	0.1203	0.2104	0.5908	0.9405	1	1	_	1	1	1	1	1	0.9724	0.8241	0.6028	0.0369	0.0108	<.0001
11	<.0001	<.0001	<.0001	<.0001	0.0062	0.0081	0.019	0.0417	0.228	0.6763	1	1	1	_	1	1	1	1	0.9974	0.9499	0.8173	0.0831	0.0266	<.0001
10	<.0001	<.0001	<.0001	<.0001	0.0027	0.0026	0.0115	0.025	0.1407	0.5369	1	1	1	1	_	1	1	1	0.9992	0.9713	0.8747	0.1037	0.037	<.0001
3	<.0001	<.0001	<.0001	<.0001	0.0038	0.0041	0.0101	0.0235	0.1603	0.576	1	1	1	1	1	_	1	1	0.9997	0.9866	0.9185	0.1487	0.0517	<.0001
21	<.0001	<.0001	<.0001	<.0001	0.0012	0.0013	0.0068	0.0149	0.0874	0.3968	1	1	1	1	1	1	_	1	0.9998	0.9886	0.9343	0.1573	0.0623	0.0001
18	<.0001	<.0001	<.0001	<.0001	0.0014	0.0015	0.0041	0.0102	0.0862	0.4097	0.9996	1	1	1	1	1	1	_	1	0.9974	0.9726	0.2444	0.0957	0.0002
12	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0014	0.0197	0.6768	0.9453	0.9724	0.9974	0.9992	0.9997	0.9998	1	_	1	1	0.9491	0.7767	0.0211
2	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0002	0.0034	0.3444	0.7151	0.8241	0.9499	0.9713	0.9866	0.9886	0.9974	1	_	1	0.9974	0.9609	0.0901
19	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0015	0.1774	0.4819	0.6028	0.8173	0.8747	0.9185	0.9343	0.9726	1	1	_	0.9999	0.9947	0.2032
20	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0027	0.0166	0.0369	0.0831	0.1037	0.1487	0.1573	0.2444	0.9491	0.9974	0.9999	_	1	0.9612
8	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0007	0.005	0.0108	0.0266	0.037	0.0517	0.0623	0.0957	0.7767	0.9609	0.9947	1	_	0.9986
6	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0001	0.0002	0.0211	0.0901	0.2032	0.9612	0.9986	_

Table 3.7: Genotype LS mean values, 95% confidence intervals (Upper and Lower Confidence Limits for individual means) and pairwise comparisons for grain TN. Overall error rate controlled by Tukey's procedure for multiple comparisons

genotype	J99C0009	4J020210-6	4J020274	5K020007	Madsen2	4J020275	4J020187	Madsen1	4J020185	5K020023	5K020095	4J020259	5K020082	5K020138	WhiteMarq2	5K020106	WhiteMarq1	Sonora	Bulk1	Bulk2	Bunyip	Idaed	Hyper	Onas
LSMean	92.0	90.1	87.2	84.9	82.3	81.8	81.2	80.8	78.1	72.7	71.7	69.4	68.2	61.7	58.4	54.7	54.2	48.3	48.0	46.7	44.8	43.7	42.6	38.6
LCL	82.7	81.1	78.3	75.4	73.1	73.0	72.3	71.9	68.3	63.4	62.5	60.1	59.0	52.5	49.2	45.9	44.8	38.9	38.8	37.8	35.4	33.5	33.4	29.8
UCL	101.4	99.2	96.0	94.5	91.6	90.7	90.0	89.8	87.9	82.0	81.0	78.6	77.5	70.9	67.6	63.6	63.5	57.8	57.3	55.6	54.3	54.0	51.8	47.4
entry	**18**	**3**	**5**	**7**	**20**	**6**	**2**	**19**	**1**	**8**	**10**	**4**	**9**	**12**	**24**	**11**	**23**	**22**	**13**	**14**	**15**	**17**	**16**	**21**
18	_	1	1	1	0.9989	0.9971	0.9932	0.9892	0.9241	0.3533	0.2658	0.1172	0.076	0.0019	0.0002	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
3	1	_	1	1	1	0.9998	0.9994	0.9989	0.9798	0.5267	0.4256	0.2152	0.1477	0.0046	0.0006	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
5	1	1	_	1	1	1	1	1	0.9997	0.8535	0.7604	0.488	0.365	0.022	0.0032	0.0002	0.0002	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
7	1	1	1	_	1	1	1	1	1	0.9868	0.9587	0.7907	0.6585	0.1022	0.0179	0.0025	0.0011	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
20	0.9989	1	1	1	_	1	1	1	1	0.999	0.9959	0.9556	0.9042	0.2359	0.062	0.0062	0.0084	0.0003	0.0001	<.0001	<.0001	<.0001	<.0001	<.0001
6	0.9971	0.9998	1	1	1	_	1	1	1	0.9994	0.9973	0.9637	0.9163	0.247	0.0626	0.0061	0.0075	0.0002	0.0001	<.0001	<.0001	<.0001	<.0001	<.0001
2	0.9932	0.9994	1	1	1	1	_	1	1	0.9998	0.9989	0.9796	0.9469	0.3038	0.0833	0.009	0.0108	0.0003	0.0002	<.0001	<.0001	<.0001	<.0001	<.0001
19	0.9892	0.9989	1	1	1	1	1	_	1	0.9999	0.9994	0.9867	0.9631	0.3443	0.1031	0.0111	0.0154	0.0005	0.0003	<.0001	<.0001	<.0001	<.0001	<.0001
1	0.9241	0.9798	0.9997	1	1	1	1	1	_	1	1	0.9999	0.9992	0.7447	0.3871	0.0814	0.1081	0.0082	0.0043	0.0016	0.0011	0.0009	<.0001	<.0001
8	0.3533	0.5267	0.8535	0.9868	0.999	0.9994	0.9998	0.9999	1	_	1	1	1	0.9939	0.8922	0.4639	0.4932	0.0741	0.0503	0.0216	0.0134	0.0118	0.002	<.0001
10	0.2658	0.4256	0.7604	0.9587	0.9959	0.9973	0.9989	0.9994	1	1	_	1	1	0.9981	0.9383	0.578	0.5756	0.0947	0.0696	0.0302	0.0175	0.0171	0.0034	0.0002
4	0.1172	0.2152	0.488	0.7907	0.9556	0.9637	0.9796	0.9867	0.9999	1	1	_	1	1	0.9933	0.8416	0.817	0.2173	0.1805	0.0884	0.0501	0.0514	0.0144	0.0008
9	0.076	0.1477	0.365	0.6585	0.9042	0.9163	0.9469	0.9631	0.9992	1	1	1	_	1	0.9985	0.9229	0.9008	0.3092	0.2689	0.1417	0.0812	0.0842	0.0275	0.0019
12	0.0019	0.0046	0.022	0.1022	0.2359	0.247	0.3038	0.3443	0.7447	0.9939	0.9981	1	1	_	1	1	1	0.9472	0.924	0.8098	0.6664	0.6226	0.3752	0.0702
24	0.0002	0.0006	0.0032	0.0179	0.062	0.0626	0.0833	0.1031	0.3871	0.8922	0.9383	0.9933	0.9985	1	_	1	1	0.9983	0.9971	0.9825	0.9353	0.9128	0.7602	0.273
11	<.0001	<.0001	0.0002	0.0025	0.0062	0.0061	0.009	0.0111	0.0814	0.4639	0.578	0.8416	0.9229	1	1	_	1	1	1	0.9999	0.9986	0.9963	0.9721	0.6499
23	<.0001	<.0001	0.0002	0.0011	0.0084	0.0075	0.0108	0.0154	0.1081	0.4932	0.5756	0.817	0.9008	1	1	1	_	1	1	1	0.9994	0.9986	0.9892	0.7574
22	<.0001	<.0001	<.0001	<.0001	0.0003	0.0002	0.0003	0.0005	0.0082	0.0741	0.0947	0.2173	0.3092	0.9472	0.9983	1	1	_	1	1	1	1	1	0.9987
13	<.0001	<.0001	<.0001	<.0001	0.0001	0.0001	0.0002	0.0003	0.0043	0.0503	0.0696	0.1805	0.2689	0.924	0.9971	1	1	1	_	1	1	1	1	0.999
14	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0016	0.0216	0.0302	0.0884	0.1417	0.8098	0.9825	0.9999	1	1	1	_	1	1	1	0.9999
15	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0011	0.0134	0.0175	0.0501	0.0812	0.6664	0.9353	0.9986	0.9994	1	1	1	_	1	1	1
17	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0009	0.0118	0.0171	0.0514	0.0842	0.6226	0.9128	0.9963	0.9986	1	1	1	1	_	1	1
16	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.002	0.0034	0.0144	0.0275	0.3752	0.7602	0.9721	0.9892	1	1	1	1	1	_	1
21	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0002	0.0008	0.0019	0.0702	0.273	0.6499	0.7574	0.9987	0.999	0.9999	1	1	1	_

Table 3.8: Genotype LS mean values, 95% confidence intervals (Upper and Lower Confidence Limits for individual means) and pairwise comparisons for total aboveground AGB production. Overall error rate controlled by Tukey's procedure for multiple comparisons

genotype	4J020210-6	J99C0009	Madsen2	4J020275	4J020274	4J020187	4J020259	Madsen1	4J020185	5K020023	5K020095	5K020007	WhiteMarq2	5K020138	5K020082	Bulk2	Bulk1	WhiteMarq1	5K020106	Bunyip	Sonora	Idaed	Hyper	Onas
LSMean	14553	14511	13002	12826	12801	12784	12379	12295	11379	11216	10990	10319	10049	9344	9237	9046	8538	8334	8158	6206	6103	5318	4996	4316
LCL	12117	11990	10743	10676	10658	10641	10228	10237	9333	9268	9080	8592	8303	7719	7632	7532	7054	6886	6793	5128	5041	4301	4128	3592
UCL	17479	17563	15736	15409	15375	15358	14982	14767	13873	13575	13301	12394	12163	11312	11180	10864	10333	10086	9798	7511	7389	6574	6047	5185
entry	**3**	**18**	**20**	**6**	**5**	**2**	**4**	**19**	**1**	**8**	**10**	**7**	**24**	**12**	**9**	**14**	**13**	**23**	**11**	**15**	**22**	**17**	**16**	**21**
3	.	1	1	1	1	1	1	0.9999	0.9827	0.9592	0.9144	0.5876	0.4756	0.1511	0.1195	0.0609	0.0186	0.0096	0.0036	<.0001	<.0001	<.0001	<.0001	<.0001
18	1	.	1	1	1	1	1	0.9999	0.988	0.9708	0.9351	0.6465	0.5327	0.1866	0.15	0.0814	0.0261	0.0141	0.0057	<.0001	<.0001	<.0001	<.0001	<.0001
20	1	1	.	1	1	1	1	1	1	1	0.9999	0.9897	0.9705	0.74	0.6779	0.5192	0.2594	0.1719	0.0939	<.0001	<.0001	<.0001	<.0001	<.0001
6	1	1	1	.	1	1	1	1	1	1	1	0.9939	0.9806	0.7809	0.7203	0.5573	0.2878	0.1889	0.1031	<.0001	<.0001	<.0001	<.0001	<.0001
5	1	1	1	1	.	1	1	1	1	1	1	0.9945	0.982	0.7864	0.7274	0.5672	0.2932	0.1945	0.1066	<.0001	<.0001	<.0001	<.0001	<.0001
2	1	1	1	1	1	.	1	1	1	1	1	0.995	0.9833	0.7948	0.7357	0.5768	0.3009	0.2004	0.1105	<.0001	<.0001	<.0001	<.0001	<.0001
4	1	1	1	1	1	1	.	1	1	1	1	0.9997	0.998	0.9279	0.8956	0.7906	0.5094	0.3786	0.2415	0.0002	0.0001	<.0001	<.0001	<.0001
19	0.9999	0.9999	1	1	1	1	1	.	1	1	1	0.9997	0.9984	0.9314	0.8994	0.7917	0.5077	0.3726	0.2336	0.0002	0.0001	<.0001	<.0001	<.0001
1	0.9827	0.988	1	1	1	1	1	1	.	1	1	1	1	0.9994	0.9985	0.9927	0.9261	0.8524	0.7282	0.0047	0.0029	0.0001	<.0001	<.0001
8	0.9592	0.9708	1	1	1	1	1	1	1	.	1	1	1	0.9997	0.9993	0.996	0.9463	0.8825	0.7678	0.005	0.0031	0.0001	<.0001	<.0001
10	0.9144	0.9351	0.9999	1	1	1	1	1	1	1	.	1	1	1	0.9999	0.999	0.9764	0.9385	0.858	0.009	0.0057	0.0002	<.0001	<.0001
7	0.5876	0.6465	0.9897	0.9939	0.9945	0.995	0.9997	0.9997	1	1	1	.	1	1	1	1	0.9994	0.9963	0.9837	0.0352	0.0234	0.0012	<.0001	<.0001
24	0.4756	0.5327	0.9705	0.9806	0.982	0.9833	0.998	0.9984	1	1	1	1	.	1	1	1	1	0.9996	0.9974	0.0822	0.0574	0.0037	0.0002	<.0001
12	0.1511	0.1866	0.74	0.7809	0.7864	0.7948	0.9279	0.9314	0.9994	0.9997	1	1	1	.	1	1	1	1	1	0.3155	0.2456	0.0245	0.0018	<.0001
9	0.1195	0.15	0.6779	0.7203	0.7274	0.7357	0.8956	0.8994	0.9985	0.9993	0.9999	1	1	1	.	1	1	1	1	0.3691	0.2905	0.0319	0.0025	<.0001
14	0.0609	0.0814	0.5192	0.5573	0.5672	0.5768	0.7906	0.7917	0.9927	0.996	0.999	1	1	1	1	.	1	1	1	0.4391	0.3514	0.0418	0.0033	<.0001
13	0.0186	0.0261	0.2594	0.2878	0.2932	0.3009	0.5094	0.5077	0.9261	0.9463	0.9764	0.9994	1	1	1	1	.	1	1	0.7962	0.7153	0.1614	0.0227	0.0002
23	0.0096	0.0141	0.1719	0.1889	0.1945	0.2004	0.3786	0.3726	0.8524	0.8825	0.9385	0.9963	0.9996	1	1	1	1	.	1	0.8901	0.8293	0.2445	0.0417	0.0004
11	0.0036	0.0057	0.0939	0.1031	0.1066	0.1105	0.2415	0.2336	0.7282	0.7678	0.858	0.9837	0.9974	1	1	1	1	1	.	0.9333	0.8866	0.2983	0.0544	0.0006
15	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0002	0.0002	0.0047	0.005	0.009	0.0352	0.0822	0.3155	0.3691	0.4391	0.7962	0.8901	0.9333	.	1	1	0.9966	0.5192
22	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0001	0.0001	0.0029	0.0031	0.0057	0.0234	0.0574	0.2456	0.2905	0.3514	0.7153	0.8293	0.8866	1	.	1	0.9989	0.6214
17	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0001	0.0001	0.0002	0.0012	0.0037	0.0245	0.0319	0.0418	0.1614	0.2445	0.2983	1	1	.	1	0.9989
16	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0002	0.0018	0.0025	0.0033	0.0227	0.0417	0.0544	0.9966	0.9989	1	.	1
21	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0002	0.0004	0.0006	0.5192	0.6214	0.9989	1	.

Table 3.9: Regression analysis of the relationship between grain N and grain yield

Variable	Parameter Estimate	Standard Error	p-value
model: grain %N = grain yield (kg/ha); R^2=0.0859			
Intercept	1.96335	0.02591	<.0001
yield	-0.00003637	0.00000617	<.0001
model: grain TN (kg/ha) = grain yield (kg/ha); R^2=0.9092			
Intercept	2.42728	1.14602	0.0349
grain %N	0.01648	0.00027381	<.0001
model: grain TN (kg/ha) = grain percent N; R^2=0.0063			
Intercept	86.91744	13.61759	<.0001
grain %N	-11.25337	7.42820	0.1307

bred genotypes because they are derived from crosses between Madsen and historic genotypes. The historic varieties had the lowest yield, grain TN and AGB production, and the organically bred genotypes show a significant increase in yield, AGB production and grain TN over the historic category The conventionally bred genotypes are derived from crosses between elite lines and this may have resulted in higher yield potential which carried over into an organic system. Similar benefits of elite germplasm were apparent in a study comparing modern maize varieties to landraces (Lafitte et al., 1997). The check genotypes Madsen and J99C0009 had the best performance in terms of yield, AGB production and grain TN. The conventionally bred genotypes were not significantly different from the check genotypes for these traits. The perennial bulk population had the highest grain %N and lowest yield among the selection categories.

All but three of 24 contrasts among selection categories were statistically significant, showing that the selection categories differed from each other individually

even when the overall ANOVA F-test was not significant. The three non-significant contrasts are also of interest. The perennial bulk population had the same grain TN as the historic category and the same AGB as the organic category. As this perennial population has a very short breeding history, these results are encouraging, especially since selection on the perennial population has been primarily for regrowth and survival over multiple seasons. With continued breeding and selection for yield, it is possible that the perennial wheat will show a similar progression as annual wheat, where modern varieties now out-yield their historic counterparts in terms of grain yield, grain TN and AGB.

Genotypic effects within categories were significant for grain %N, grain yield, grain TN and AGB. This shows that significant variation exists within categories as well as among categories, and selection on these traits in an organic system would be effective. In the study of maize landraces, Lafitte et al. (1997) also found large genetic variation in the proportion of plant N found in the grain (nitrogen harvest index, NHI), total N uptake and grain %N under low N conditions and suggested that selection under low N for these traits may result in greater genetic gains for these environments. Of the three SPAD readings, the SPAD3 reading would be most useful for selecting genotypes with higher yield, grain TN and grain %N, because it appears that higher SPAD readings at anthesis are predictive of higher values for these traits at maturity. SPAD2 was also significant in the case of grain %N, and if resources were available, could be used in addition to SPAD3 in a breeding program.

However, it is possible that high SPAD readings are correlated to higher soil moisture status or N levels rather than to genotypic effects so these should be used with caution (Chapter 5). Environmental factors were also significant, and much of this can be attributed to year to year variation and interaction effects. Interactions due to year were important for all four traits in both the category and the genotype model. In contrast, interactions between category and location were not significant, and interactions between genotype and location were complex, with grain %N having a significant genotype by location interaction, grain yield and AGB having a significant three-way interaction and grain TN not significant for either the two way or three way interaction at the p=0.05 level. It appears that yearly variation in growing conditions has a larger effect on these traits than the location effect, however, it is also clear the genotype by environment interactions are complex and difficult to predict. The presence of significant interactions and block effects means that trial results will be more reliable when replicated over several years and locations.

3.4.2 Relationship between grain N and yield

Oury and Godin (2007) stated that grain TN takes both yield and grain %N into account, but is more strongly influenced by yield than by %N, so low yielding genotypes with high %N will not be ranked highly for grain TN. Our results confirm this expectation, as a regression of grain TN on grain yield has an R^2 of 0.9092, while a regression of grain TN on grain %N has an R^2 of 0.0063. Positive residuals

from a regression of grain %N on yield indicate lines with higher protein than expected for their yield, and negative residuals indicate lines with lower protein than expected. We did not identify any lines with standardized residuals greater than two standard deviations (+/-1.96) away from the regression line, possibly because most genotypes were soft white wheat and we had a limited number of locations and years.

For soft white wheat, high grain protein is not required for marketing as it is for hard red or white wheat, and in organic systems, lines able to yield well at lower protein concentrations may be advantageous. If end-use and mineral nutritional quality do not suffer, using negative GPD as a selection criteria as well as yield under low N conditions could be used to breed wheat for lower grain N requirements. Interestingly, both quality checks used by Oury and Godin (2007) had negative GPD, so it appears that high protein with respect to yield is not necessarily an indicator of end-use quality. A study specifically on the relationship between end-use quality and GPD, would be useful in determining the reliability of GPD as a selection criterion.

3.5 Conclusions

Conducting this study in an organic system using genetically diverse material, including annual and perennial wheats, provided information about genetic differences that will be used in the breeding programs to select for high NUE under conditions of relatively low available N. SPAD meter readings could be useful when

taken immediately post-anthesis and incorporated into an overall evaluation of breeding lines. Regression analysis to calculate GPD was not useful for this set of genotypes, possibly due to a narrow range of grain protein concentrations. Genotypes with high relative performance for each selection category can be identified from the LS means analysis of AGB, grain TN, grain %N and grain yield. These genotypes show promise for adaptation to organic systems and could be both advanced in yield trials and crossed to combine favorable aspects of each. The fact that location effects were less often significant than year effects indicates that it may be possible to breed varieties for organic systems within ecologically similar regions, such as the relatively high rainfall zone of eastern Washington. Breeding wheat with superior performance in organic systems may help wheat farmers transition to more sustainable fertility management without economic losses.

Acknowledgments

The authors would like to thank Steven Lyon, Kerry Balow and Meg Gollnick for extensive help in the management of this experiment. David Uberuagua, Margaret Davies and Ron Bolton provided technical expertise in the analysis of soil and plant samples. Richard Alldredge was of great help in the statistical analysis. This work was funded by the National Science Foundation under a Graduate Research Fellowship and by a Graduate Research Fellowship of The Land Institute, Salina, KS. Any opinions, findings, and conclusions or recommendations expressed in

this material are those of the authors and do not necessarily reflect the views of the National Science Foundation or the Land Institute.

Bibliography

Allan R.E., Peterson, Jr. C.J., Rubenthaler G.L., Line R.F., Roberts D.E., 1989. Registration of 'Madsen' wheat. Crop Science 29, 1575.

Bertin P., Gallais A., 2000. Genetic variation for nitrogen use efficiency in a set of recombinant maize inbred lines I. Agrophysiological results. Maydica 45, 55–66.

Chapin III F.S., 1980. The mineral nutrition of wild plants. Annual Review of Ecology and Systematics 11, 233–260.

Foulkes M.J., Sylvester-Bradley R., Scott R.K., 1998. Evidence for differences between winter wheat cultivars in aquisition of soil mineral nitrogen and utilization of applied fertilizer nitrogen. Journal of Agricultural Science 130, 29–44.

Fowler D.B., Brydon J., Darroch B.A., Entz M.H., Johnston A.M., 1990. Environment and genotype influence on grain protein concentration of wheat and rye. Agronomy Journal 82, 655–664.

Gáborčík N., 2003. Relationship between contents of chlorophyll (a+b)(SPAD values) and nitrogen of some temperate grasses. Photosynthetica 41, 2855–287.

Giunta F., Motzo R., Deidda M., 2002. Spad readings and associated leaf traits in durum wheat, barley and triticale cultivars. Euphytica 125, 197–205.

Huggins D.R., Pan W.L., 2003. Key indicators for assessing nitrogen use efficiency in cereal-based agroecosystems. Journal of Crop Production 8, 157–185.

Jiang Z., Hull R.J., 1998. Interrelationships of nitrate uptake, nitrate reductase, and nitrogen use efficiency in selected Kentucky Bluegrass cultivars. Crop Science 38, 1623–1632.

Keeney D.R., Nelson D.W., 1982. Nitrogen-inorganic forms. In: Weaver R.W., et al. (eds.), Methods of Soil Analysis Part 2: Chemical and Microbiological Properties. ASA-SSSA, Madison, WI.

Lafitte H.R., Edmeades G.O., Taba S., 1997. Adaptive strategies identified among tropical maize landraces for nitrogen-limited environments. Field Crop Research 49, 187 – 204.

Moll R.H., Kamprath E.J., Jackson W.A., 1982. Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization. Agronomy Journal 74, 562–564.

Morris C.F., Paulsen G.M., 1985. Development of hard winter wheat after anthesis as affected by nitrogen nutrition. Crop Science 25, 1007–1010.

Murphy K.M., Campbell K.G., Lyon S.R., Jones S.S., 2007. Evidence of varietal adaptation to organic farming systems. Field Crops Research 102, 172–177.

Oury F., Godin C., 2007. Yield and grain protein concentration in bread wheat: how to use the negative relationship between the two characters to identify favourable genotypes? Euphytica 157, 45–57.

Sinclair T.R., 1998. Historical changes in harvest index and crop nitrogen accumulation. Crop Science 38(3), 638–643.

United States Department of Agriculture, 2000. National Organic Program: Final Rule. 7 Code of Federal Regulations Part 205.

Van Sanford D.A., MacKown C.T., 1987. Cultivar differences in nitrogen remobilization during grain fill in soft red winter wheat. Crop Sci. 27, 295–300.

Vidal I., Longeri L., Hétier J.M., 1999. Nitrogen uptake and chlorophyll meter measurements in spring wheat. Nutrient cycling in agroecosystems 55, 1–6.

Watson C.A., Atkinson D., Gosling P., Jackson L.R., Rayns F.W., 2002. Managing soil fertility in organic farming systems. Soil Use and Management 18(S1), 239–247.

Chapter 4

Assessing genetic variation for plant N, biomass production and grain traits in annual and perennial wheat grown with organic fertilizer

Abstract

Nitrogen (N) cycling and availability in organic systems differs from that in conventional systems. Little research has been done on genetic differences in N uptake and partitioning under conditions of organic fertility management. The identification of genotypes or genetic sources of traits related to higher nitrogen use efficiency (NUE) would help in breeding varieties with better adaptation to organic agricultural systems. In this study, a diverse group of wheat types, including historic and modern spring wheat varieties, perennial breeding lines and disomic chromosome addition lines (*Triticum aestivum*L./*Thinopyrum elongatum* were tested in

greenhouse conditions. All genotypes were grown with organic fertilizer and sampled for aboveground biomass (AGB) and % nitrogen (N) at the physiological stages 6-leaf, anthesis and maturity. AGB, total N and %N were compared at the 6-leaf and anthesis stage, and at maturity a number of traits related to N uptake, partitioning and efficiency were compared among genotypes. Significant genetic differences among lines were found for nearly all variables tested. Among the annual lines, historic varieties had greater AGB production but modern varieties had higher %N in vegetative growth stages. At maturity, modern varieties had greater straw %N and harvest index, and historic varieties had greater straw weight and AGB, with no significant differences for other measured variables. Historic varieties may possess useful traits related to N use because they were able to produce more vegetative AGB at the same N supply without sacrificing grain weight. Comparisons of genotypes in the perennial and addition lines identified certain genotypes with good performance for single traits such as grain %N that could be crossed to combine these traits in varieties of perennial wheat.

4.1 Introduction

In developed agricultural systems, most plant nitrogen (N) needs are met through the addition of synthetic fertilizers. Synthetic N fertilizers are produced from non-renewable natural gas, and environmental concerns related to the

over-application of N are growing. Many farmers are seeking to reduce their fertilizer inputs, and the use of organic practices is increasingly important. For both environmental and economic reasons, crop varieties with more efficient N uptake and partitioning will be critical to sustaining agriculture. Studying nitrogen use efficiency (NUE) under low N and organic conditions is an important step in the development of varieties with high yields and adequate protein when faced with available N limitations. Using genetically diverse material, including annual and perennial wheats, should provide information about genetic differences that can be used in breeding programs to select for high NUE under conditions of relatively low available N.

Current perennial wheat breeding lines and historic wheat varieties yield less than modern annuals under field conditions, but may have traits important to efficient N use under organic and N limited conditions (Foulkes et al., 1998; Lafitte et al., 1997). In developed agricultural systems, breeding programs for cereal grains are often conducted in highly controlled environments with high levels of fertility provided through synthetic fertilizers. For the purpose of this study, varieties released before 1955 were considered to be historic. This date corresponds roughly to when breeding programs began to use synthetic fertilizer in selection nurseries (Murphy et al., 2007). Perennial breeding lines are the result of crosses between annual wheat and wild wheat grasses (*Thinopyrum* spp.) which evolved in prairie ecosystems (Piaskowski, 2006). In such environments, inorganic N pools are

extremely small and plant species must make maximal use of available N. Traits that increase N conservation within the plant and/or the efficiency of internal N cycling are expected to be of benefit in low-input systems. Because low-input and organic systems are more similar to natural ecosystems than conventional systems are, traits related to NUE in an ecological context may be of greater importance (Chapter 2).

While historic and modern annual wheat varieties likely differ in terms of their N dynamics, relative performance in terms of N uptake and N utilization may change depending on the environmental context. In particular, the higher harvest index (HI) of modern varieties means that a greater proportion of the total AGB is contained in the grain, which has a higher N concentration than straw. Because breeders have been directly and indirectly selecting for higher HI and higher grain N content, modern varieties may have the ability to take up more N and translocate it to the grain than historic varieties (Sinclair, 1998).

However, modern varieties may be less able to effectively use N in low-input and organic systems. It is likely that modern varieties have been selected for increased rates of N uptake during the period following N fertilizer application. Historic varieties developed before breeding programs commonly used synthetic fertilizers may be better adapted to the slower mineralization rates of organic N forms. A study comparing historic and modern varieties showed that historic varieties were better able to extract and use soil N when synthetic fertilizer was absent (Foulkes et al., 1998). In contrast, recent cultivars were more responsive to

high N supplies. The authors stated that the decrease in soil N uptake capacity may have been because of less vigorous early growth and rooting in semi-dwarf cultivars (Foulkes et al., 1998). A similar study comparing tall and semi-dwarf varieties showed that yields were similar under high-N conditions, but tall genotypes yielded more in low-N situations (Morris and Paulsen, 1985).

These differences indicate that modern varieties may not be well-suited to low-input or organic systems. While they may have yields equal to or greater than historic varieties, this does not mean that conventionally bred modern varieties make optimal use of organic N sources. Conventional breeding methods are unlikely to have improved the adaptation of modern varieties to low N conditions, even if there are spillover benefits in terms of yield (Lafitte et al., 1997). Because low-input and organic systems are highly complex and rely almost entirely on biological cycling of nutrients, traits related to improving N use may be quite different than those selected for in conventional systems. Significant changes in rank have been observed in variety yields in side-by-side comparisons of organic and conventional systems (Murphy et al., 2007). This is an indication that to optimize performance in low-input and organic systems, selection must be conducted in those systems. An understanding of the N dynamics of historic and modern varieties under organic N fertility management can help in selecting parents and choosing a breeding strategy to take advantages of traits in both historic and modern germplasm.

The objectives of these experiments were to compare N uptake and partitioning

in annual historic and modern spring wheat varieties, perennial wheat genotypes, and a series of *Triticum aestivum*/*T. elongatum* chromosome addition lines. The chromosome addition lines were used to determine the effects of perennial chromosomes on N uptake and partitioning in a spring wheat genetic background.

Understanding variation in these traits in both annual and perennial germplasm and the potential effects of individual perennial chromosomes will aid breeders in incorporating traits important to efficient N use into perennial and annual wheat varieties. Knowledge of which chromosomes have a significant effect on traits related to N use efficiency will contribute to studies on quantitative trait loci and to the eventual mapping of genes of importance.

4.2 Materials and Methods

4.2.1 Germplasm

Annual genotypes were selected from field evaluations to represent a diverse range of performance in terms of yield and grain protein levels. Annual genotypes used are listed in table 4.2.1. Perennial wheat breeding lines from the Washington State University Winter Wheat Breeding Program (WSU WW) were also tested, using the same methods (Table 4.2. For a full discussion of the genetic composition of the perennial lines, see Piaskowski (2006). A series of chromosome addition lines

were tested to determine the effect of perennial chromosomes on NUE. *Th. elongatum* is a perennial wild relative of wheat that contains the E genome. A complete amphiploid and seven chromosome addition lines of Chinese Spring (*T. aestivum* L.) and *Th. elongatum* chromosomes (Dvorak and Knott, 1974) were tested along with *Th. elongatum* and Chinese Spring (Table 4.3).

4.2.2 Experimental Design

The experiments were run as separate randomized complete block (RCBD) experiments in the same greenhouse bay. Each experiment was run three times in succession (three cycles), so that environmental variation in the greenhouse could be assessed in the analysis. The annual experiment cycle one was planted September 30, 2005, cycle two was planted March 7, 2005 and cycle three was planted July 18 2006. The addition line experiment cycle one was planted March 7, 2006, cycle two was planted July 28, 2006 and cycle three was planted September 9, 2006. The perennial experiment cycle one was planted February 16, 2006, cycle two was planted November 2, 2006 and cycle three was planted January 23, 2007. Perennial wheat breeding lines and *Th. elongatum* were vernalized in peat pellets for 8 weeks, then planted in three liter pots in the greenhouse bay and fertilized (see below for fertilizer treatment). The greenhouse is set at a 16 h photoperiod, with at temperature of 21-24 C, with lights that come on when outside light intensity falls below $300\,mmol/m/s^2$. Supplemental light provides about $400\,mmol/m/s^2$.

Table 4.1: Annual spring wheat genotypes used in the greenhouse study including pedigrees, date of release and market classes

Genotype	Pedigree	Released	Market Class
Alpowa	Fielder/Potam 70//Walladay/3/Walladay/Potam 70	1994	Soft white spring
Arco	Arcadia/Hard Federation	1928	Soft white winter
Bunyip	Rymer/Maffra	1901	Soft white spring
Canus	Marquis/Kanred	1935	Hard red spring
Currawa	Northern Champion/Cretan//Little Club	1912	Soft white spring
Idaed	Sunset/Boadicea	1938	Soft white spring
Onas	Federation/Tarragon	1915	Soft white spring
Pacific Bluestem	Collected from Oregon, United States	?	Soft white spring
Penawawa	Potam 70/Fielder	1985	Soft white spring
Pilcraw	Field selection, California	1917	Soft white spring
Red Fife	Collected from Ontario, Canada	1842	Hard red spring
Scarlet	Tifton 3725/Walladay/3/Fielder//Bronz/Koeltz-7941s.5/5/Henry/ Karn90, S.90//Burt/Onas 52/3/ Lemhi66/4/ Yaktana54A*4//Norin 10/Brevor 14/6/Tifton3725/ Walladay/3/Fielder//Bronz/Koelt-7941S.5/7/Tecumseh/5/Tifton 3725/Walladay/4/Bezostaja 1// (14x53-101)/Burt#4/3/ Burt/Kenya Farmer 70136	1999	Hard red spring
Sonora	Collected from Durango, Mexico	1907	Soft white spring
Spinkcota	Preston sel./red durum// Preston sel.	1944	Hard red spring
Surprise	Collected from Washington, United States	1870	Soft white spring
Wakanz	K78504/K79129-33//K7806645	1987	Soft white spring
Wawawai	ID0046/7/ID0045/6/2*A6596S-A-21-1/5/2*A6535S-443-107/4/A6316 7S-A-1-59-2-2/3/Thew/Federation//A63166S-A-2-8/8/Potam70/ Fielder/5/Tifton3725/Walladay/3/ Fielder/Brons/Koelz794S70-5/4/ Lemhi66/3/Yaktana54A*4//Norin10/Brevor/4/IDO065/Potam 70	1994	Soft white spring
Westbred Express	private variety	1991	Hard red spring
White Federation	selection from Hard Federation	1915	Soft white spring
White Marquis	mutant in Marquis	1923	Hard white spring
Zak	Pavon'S'/5/PI167822/CI13438113-6//Idaed/Marfed68-5/4/Lemhi66/3/ Yaktana54A*4/Norin10/Brevor/6/Walladay/7/PI506355/8/Treasure	2002	Soft white spring

Marquis pedigree: Hard Red Calcutta/Red Fife, released 1910
Pedigree information from USDA, ARS, National Genetic Resources Program. Germplasm Resources Information Network - (GRIN). [Online Database] National Germplasm Resources Laboratory, Beltsville, Maryland. Available: http://www.ars-grin.gov/npgs/searchgrin.html (01 January 2007)

Table 4.2: Perennial wheat breeding lines from the WSU WW used in the greenhouse study

Perennial line	Pedigree
03JP004	Madsen//Chinese Spring/*Thinopyrum sp.*
03JP016	Madsen//Chinese Spring/*Thinopyrum sp.*
03JP019	Madsen//Chinese Spring/*Thinopyrum sp.*
03JP022	Madsen//Chinese Spring/*Thinopyrum sp.*
03JP026	Madsen//Chinese Spring/*Thinopyrum sp.*
03JP036	Madsen//Chinese Spring/*Thinopyrum sp.*
03JP039	Madsen//Chinese Spring/*Thinopyrum sp.*
PI550713	x *Agrotriticum sp. bulk population*
SS259	*T.vulgare/Agropyron elongatum* P315 (50)//*T.vulg*

Table 4.3: Chinese Spring/*Th. elongatum* chromosome addition lines used in the study

Genotype	Pedigree	Characterization
Chinese Spring	Collection, CItr 14108	Annual $2n = 6x = 42$
AgCS	Chinese Spring/*Th. elongatum*	Amphiploid $2n = 8x = 56$
CS+1E	Chinese Spring/*Th. elongatum*	Annual single disomic chromosome addition
CS+2E	Chinese Spring/*Th. elongatum*	Annual single disomic chromosome addition
CS+3E	Chinese Spring/*Th. elongatum*	Annual single disomic chromosome addition
CS+4E	Chinese Spring/*Th. elongatum*	Perennial single disomic chromosome addition*
CS+5E	Chinese Spring/*Th. elongatum*	Annual single disomic chromosome addition
CS+6E	Chinese Spring/*Th. elongatum*	Annual single disomic chromosome addition
CS+7E	Chinese Spring/*Th. elongatum*	Annual single disomic chromosome addition
Th. elongatum	PI578686^{+}	Perennial $2n = 2x = 14$
All genotypes were obtained from Dr. J. Dvorak, University of California at Davis		
See Lammer et al. (2004) for a discussion of the perenniality of CS+4E		
$^{+}$*Th. elongatum* Host D.R. Dewey POACEAE, cultivar "Orbit" developed in Saskatchewan, Canada		

Nighttime temperatures are set at 15-18 C.

Each genotype was replicated nine times, with each plant grown separately in a three liter pot with standard potting soil (Sunshine Mix LC1). All plants received the same treatment, with 40 g Perfect Blend organic fertilizer (4-4-4) added to the potting soil before planting. All aboveground plant material in three replicates was sampled at the six-leaf growth stage (6leaf), anthesis, and maturity. Just prior to sampling, leaf chlorophyll content was measured using a SPAD chlorophyll meter (Minolta Co, Japan) for the experiments with chromosome addition lines and perennial lines at the 6leaf and anthesis stage, and for the perennial experiment at the maturity stage if there were still green leaves at physiological maturity. The average of four readings along the flag leaf of the most advanced head was recorded. The plant samples were then analyzed for tissue %N. Plants were air-dried for 48 h at 60 C. Dry AGB was weighed, then ground through a 2 mm screen using a Wiley Mill (Thomas Wiley Co.). At maturity, grain and straw samples were analyzed separately. Plant heads were weighed and then threshed to obtain grain samples, which were weighed and ground to flour using a Cyclone Mill (UDY Corporation, Ft. Collins, CO). Chaff weight was obtained by difference and added to straw weight. For all plant tissue, a sample weighing 0.2500-3.0000 g was measured out and analyzed for %N using a CNS-2000 Elemental Analyzer (LECO corporation, St. Joseph, MI). Total aboveground plant N (TN) was calculated as (%N$*$AGB) and this measure was used as the N uptake for each sample. N partitioning was calculated at

maturity by analyzing grain and vegetative AGB separately for N content.

4.2.3 Analysis

ANCOVA models in SAS (SAS Institute, Cary, NC) were used to analyze each of the three greenhouse experiments. The dependent variables were AGB, total aboveground plant N (TN), and plant %N at 6leaf and anthesis, and grain %N, grain TN, grain weight, straw %N, straw TN, straw weight, total AGB, N uptake (total aboveground plant N; N up), N utilization efficiency (grain weight/ total plant N; N ut), N harvest index (grain TN/total aboveground plant N; NHI) and harvest index (HI) at the maturity sampling date. SPAD meter readings and the number of days after planting that the sample was taken (DAP) were tested as covariates and retained if significant. Year of release for the variety and market class were also tested in the annual experiment but were not found to be significant. All significance tests were perfomed at the $p<0.05$ level. The variance increased as %N increased for straw %N in the annual varieties, for straw %N and straw TN in the chromosome addition lines and for anthesis%N, straw %N and straw TN for the perennial breeding lines, so a logarithmic transformation was used to stabilize the variance.

For the analysis of annual genotypes, the genotypic variation was assessed separately from the category (historic or modern). The least squared (LS) mean value of each category was calculated and compared for the measured variables. The

LS mean value of each genotype was also calculated and Tukey's method was used for all pairwise comparisons.

4.3 Results

4.3.1 Annual varieties

The covariate DAP was significant at 6leaf and anthesis, except for TN at anthesis. At maturity, DAP was dropped and an ANOVA model was used. The DAP covariate slope was small for all models where it was significant. DAP was negatively correlated with %N at the 6leaf and anthesis and positively correlated with AGB at both stages and with TN at the 6leaf stage (Table 4.4 and Table 4.5). The varieties Alpowa and Wawawai had poor germination and survival so Alpowa was excluded from the 6leaf stage, and both Alpowa and Wawawai were excluded from the anthesis stage.

The environmental component of variation (differences due to the experimental cycle) was generally significant in both the genotype and the category model. As the greenhouse is already a controlled environment, the main purpose of assessing the environmental component of variation is to exclude this component from the analysis of the genetic component of variation. However, genotype by environment interactions were also present for many measured variables (Table 4.4 and Table 4.5).

Table 4.4: Greenhouse ANCOVA results for six leaf and anthesis stages: p-values for significance tests and LS means for historic and modern annual categories

	Dependent Variable					
	6-leaf stage			anthesis stage		
	Percent N	total N (g/plant)	AGB (g/plant)	percent N	total N (g/plant)	AGB (g/plant)
Annual varieties comparison of category LS mean values						
historic	5.602	0.125	2.33	1.273	0.4098	35.26
modern	5.750	0.0923	1.68	1.561	0.4209	29.97
p-value	0.0499	<.0001	<.0001	<.0001	0.3876	<.0001
Annual varieties: Category model						
DAP	0.0014	<.0001	<.0001	<.0001	-	<.0001
cycle	0.0207	0.0544	0.0088	0.0042	0.0385	0.0535
category	0.2138	0.0837	0.0273	0.0410	0.7088	0.1006
category*cycle	0.3089	0.1439	0.6236	0.3689	0.0192	0.1501
DAP slope	-0.01642	0.002253	0.05476	-0.0148	-	0.3657
Annual varieties: Genotype model						
DAP	0.0074	<.0001	<.0001	0.0055	-	0.0180
cycle	<.0001	<.0001	<.0001	<.0001	<.0001	0.0010
genotype	0.1624	0.0347	0.0106	0.0290	0.1903	0.0054
genotype*cycle	0.0596	<.0001	0.0053	<.0001	0.0033	0.0045
DAP slope	-0.02122	0.0054376	0.1124	-0.0063	-	0.1324
Addition lines						
spad	0.0343	-	-	-	-	-
dap	-	-	-	<.0001	-	<.0001
cycle	0.4799	<.0001	<.0001	<.0001	0.0004	0.0004
genotype	<.0001	0.0308	0.0234	0.5995	0.5566	0.4475
genotype*cycle	0.0003	0.1127	0.1596	<.0001	0.0005	0.0048
spad*genotype	<.0001					
covariate slope	0.0040	-	-	-0.0268	-	0.7023
Perennial lines						
spad	-	-	-	0.0007	-	-
dap	-	-	-	0.0300	-	0.0023
cycle	0.0267	<.0001	<.0001	<.0001	<.0001	0.0632
genotype	0.4629	0.3994	0.3304	0.3507	0.3625	0.6035
genotype*cycle	0.0201	0.1521	0.1726	<.0001	0.2272	0.0044
SPAD slope	-	-	-	0.0219	-	0.4512
DAP slope	-	-	-	-0.0082	-	-

Table 4.5: Greenhouse ANCOVA results for maturity: p-values for significance tests and LS means for historic and modern annual categories

	Dependent Variable										
	grain %N	grain TN	grain wt	straw %N	straw TN	straw wt	AGB	N up	N ut	HI	NHI
Annual varieties category category LS means comparison											
historic	1.970	0.3894	20.01	0.3185	0.1129	34.58	54.59	0.5023	42.05	0.3655	0.7582
modern	1.934	0.3827	20.77	0.4000	0.1173	28.30	49.07	0.5000	43.79	0.4229	0.7561
p-value	0.6087	0.6141	0.4205	<.0001	0.3803	<.0001	0.0018	0.8482	0.2786	<.0001	0.8876
Annual varieties: Category model											
cycle	0.0301	0.0376	0.4284	0.0096	0.0522	0.1010	0.4561	0.0169	0.0512	0.2041	0.5222
category	0.7848	0.8685	0.7658	0.0400	0.6995	0.0436	0.1605	0.9439	0.6195	0.1858	0.9377
category*cycle	0.0694	0.0009	0.0043	0.2673	0.0221	0.3198	0.1242	0.0036	0.0327	0.0067	0.0792
Annual varieties: Genotype model											
cycle	<.0001	<.0001	0.0012	<.0001	<.0001	0.0007	0.2092	<.0001	<.0001	<.0001	0.1094
genotype	0.0107	0.2937	0.0578	0.0005	0.0157	0.0005	0.0091	0.4673	0.0281	0.0033	0.1133
genotype*cycle	0.0195	0.0029	0.0126	0.0569	0.2037	0.1345	0.0591	0.0137	0.0519	0.0333	0.0183
Addition Lines											
DAP	0.0035	-	-	0.0133	0.0003	-	-	-	-	-	-
cycle	0.0056	0.0011	0.0505	<.0001	0.0766	0.0038	0.0216	<.0001	0.1916	0.0080	0.2042
genotype	0.0248	0.0216	0.0102	0.6352	0.7724	0.0376	0.3250	0.0664	0.0221	0.0048	0.0092
genotype*cycle	0.0062	0.0137	0.0476	0.0071	0.0009	0.0740	0.0227	0.0373	0.0307	0.0152	0.0223
DAP slope	0.0596	-	-	0.0177	0.0293	-	-	-	-	-	-
Perennial lines											
DAP	-	-	-	-	0.0118	0.0411	-	-	-	-	-
cycle	0.1619	<.0001	<.0001	0.0060	0.0578	<.0001	<.0001	<.0001	0.6912	<.0001	0.0160
genotype	0.0205	0.4174	0.2458	0.0120	0.1038	0.0025	0.0110	0.1314	0.1337	0.4465	0.2703
genotype*cycle	0.0446	0.4684	0.0060	0.0326	0.0046	0.8380	0.4847	0.5868	0.1628	0.0197	0.0215
DAP slope	-	-	-	-	0.0119	0.2777	-	-	-	-	-
all measurements in grams. TN= total N; N up= N uptake; N ut = N utilization efficiency											

These environmental effects could have been due to conducting the cycles at different points during the year, as the supplemental light does not entirely compensate for natural changes in day length, and temperature fluctuations can be greater in the summer months when high outside temperatures and incoming light makes it more difficult to keep the greenhouse bays in the 21-24 C range. Genotypic main effects were significant for all measured variables except %N at 6leaf, TN at anthesis and grain TN, N up and NHI at maturity. The category (historic or modern) was significant for AGB at 6leaf and straw %N and straw weight at maturity.

Comparison of LS mean values for historic and modern annual genotypes (also in Table 4.4 and Table 4.5) showed that historic genotypes had greater TN and AGB at 6leaf but modern genotypes had higher %N. At anthesis, there was no difference in TN, with historic genotypes still having greater AGB and modern genotypes having greater %N. At maturity, modern varieties had greater straw %N and HI, and historic varieties had greater straw weight and AGB, with no significant differences for other measured variables.

LS mean values for individual annual genotypes are shown in Table 4.6, Table 4.7 and Table 4.8. For 6leaf AGB, Idaed was greater than Red Fife, Westbred Express, Penawawa and Wawawai. For 6leaf %N there were no significant differences among genotypes. For 6leaf TN, Idaed was greater than Red Fife, Westbred Express, Penawawa and Wawawai. Penawawa was less than Idaed, Surprise, Pilcraw and Sonora.

For AGB at anthesis, Pacific Bluestem and Arco were greater than Idaed, Spinkcota, Red Fife, Canus, White Marquis, Penawawa, Currawa, Wakanz, Scarlet, Surprise, White Federation and Zak. White Federation and Zak were also less than Pilcraw, Bunyip, Onas and Sonora. For anthesis %N, Zak was greater than White Marquis, Spinkcota, Onas, Westbred Express, Surprise, Pacific Bluestem, Pilcraw, Sonora, Bunyip, and Arco. Currawa was greater than Onas, Pacific Bluestem, Pilcraw, Sonora, Bunyip and Arco. Penawawa and White Federation were greater than Pacific Bluestem, Pilcraw, Sonora, Bunyip, and Arco. Idaed, Canus and Wakanz were greater than Pilcraw, Sonora, Bunyip and Arco. For anthesis TN there were very few significant differences, with Idaed greater than Sonora, Red Fife and White Federation.

There were significant differences among genotypes at maturity for each trait except N uptake. For grain %N, Westbred Express was greater than Zak, Bunyip, Onas, Penawawa, Pilcraw and Wawawai. Pacific Bluestem and Sonora were greater than Pilcraw and Wawawai and Idaed was greater than Wawawai. For grain TN, Pacific Bluestem was less than Idaed, White Federation and White Marquis. For grain weight, Wawawai was greater than Westbred Express, Red Fife, Sonora and Pacific Bluestem. Pacific Bluestem was also less than Onas, Penawawa, Bunyip and Zak. For N utilization efficiency Wawawai was greater than Sonora, Westbred Express and Pacific Bluestem. Penawawa was greater than Westbred Express and Pacific Bluestem. For HI, Wawawai and White Federation were greater than Sonora

and Pacific Bluestem. Pacific Bluestem was also less than Penawawa, Alpowa, Scarlet, Zak, Idaed, Westbred Express, Currawa, Onas, Bunyip, Wakanz and White Marquis. For NHI, there were not many significant differences. White Federation was greater than Pacific Bluestem and Wakanz, and Idaed was greater than Wakanz.

There were several significant differences for traits related to straw at maturity. For straw weight, Pacific Bluestem was greater than Red Fife, Wakanz, White Federation, Currawa, Idaed, Alpowa, Westbred Express and Scarlet. Pilcraw was greater than White Federation, Currawa, Idaed, Alpowa, Westbred Express and Scarlet. Arco and Canus were greater than Westbred Express and Scarlet. Spinkcota and Bunyip were both greater than Scarlet. For straw %N Wakanz was greater than Penawawa, Pilcraw, Wawawai, White Marquis, Arco, Sonora, Spinkcota, Onas, Bunyip, Red Fife and Canus. Alpowa was greater than Sonora, Spinkcota, Onas, Bunyip, Red Fife and Canus. Westbred Express was greater than Pilcraw, Wawawai, White Marquis, Arco, Sonora, Spinkcota, Onas, Bunyip, Red Fife and Canus. There were fewer significant differences for straw TN, with Wakanz being greater than White Federation, Scarlet, Wawawai, Red Fife and Idaed. For total aboveground plant AGB, Pilcraw, Bunyip and Onas were greater than Scarlet and Westbred Express, and Canus was greater than Westbred Express.

Table 4.6: Genotype LS mean values and 95% confidence intervals for individual means (Upper and Lower Confidence Limits) at 6leaf and anthesis for AGB, %N and TN

6leaf

genotype	weight (g)	UCL	LCL
Idaed	3.20	2.65	3.75
Sonora	2.71	2.16	3.26
Pilcraw	2.64	2.08	3.20
Bunyip	2.58	2.02	3.14
Surprise	2.50	1.94	3.05
Onas	2.28	1.57	3.00
Scarlet	2.28	1.73	2.83
White Marquis	2.22	1.67	2.77
Currawa	2.20	1.63	2.77
Arco	2.10	1.46	2.74
White Federation	2.08	1.48	2.67
Zak	2.03	1.43	2.62
Spinkcota	1.93	1.38	2.48
Canus	1.88	1.28	2.47
Wakanz	1.85	1.29	2.40
Pacific Bluestem	1.80	1.16	2.44
Red Fife	1.72	1.14	2.29
Westbred Express	1.60	1.05	2.15
Penawawa	1.52	0.967	2.07
Wawawai	1.15	0.407	1.90

genotype	%N	UCL	LCL
White Marquis	5.926	5.635	6.216
Currawa	5.914	5.615	6.214
Canus	5.848	5.533	6.164
Wakanz	5.789	5.495	6.083
White Federation	5.782	5.467	6.097
Spinkcota	5.771	5.480	6.061
Westbred Express	5.768	5.478	6.058
Arco	5.755	5.419	6.091
Surprise	5.696	5.404	5.988
Wawawai	5.674	5.281	6.067
Scarlet	5.631	5.339	5.923
Penawawa	5.627	5.337	5.918
Onas	5.583	5.206	5.960
Red Fife	5.570	5.266	5.874
Pilcraw	5.502	5.209	5.796
Sonora	5.502	5.212	5.792
Bunyip	5.441	5.146	5.735
Zak	5.440	5.127	5.754
Idaed	5.336	5.045	5.626
Pacific Bluestem	5.239	4.904	5.574

genotype	TN (g)	UCL	LCL
Idaed	0.160	0.137	0.184
Surprise	0.140	0.116	0.164
Pilcraw	0.139	0.115	0.163
Sonora	0.138	0.114	0.162
Bunyip	0.134	0.110	0.158
White Marquis	0.129	0.106	0.153
Scarlet	0.127	0.103	0.151
Onas	0.125	0.094	0.156
Currawa	0.125	0.100	0.149
White Federation	0.118	0.0922	0.144
Arco	0.111	0.0833	0.138
Spinkcota	0.107	0.0836	0.131
Canus	0.106	0.0807	0.132
Wakanz	0.102	0.0777	0.126
Zak	0.101	0.0754	0.127
Pacific Bluestem	0.0937	0.0663	0.121
Red Fife	0.0898	0.0649	0.115
Westbred Express	0.0894	0.0657	0.113
Penawawa	0.0717	0.0479	0.0954
Wawawai	0.0666	0.0344	0.0987

anthesis

genotype	weight (g)	UCL	LCL
Pacific Bluestem	45.96	40.83	51.09
Arco	45.76	40.86	50.66
Pilcraw	39.22	34.98	43.46
Bunyip	38.14	33.89	42.39
Onas	37.73	33.16	42.30
Sonora	37.48	33.25	41.70
Westbred Express	35.30	30.58	40.02
Idaed	33.08	28.82	37.34
Spinkcota	31.20	26.27	36.12
Red Fife	31.16	26.92	35.41
Canus	31.14	26.88	35.40
White Marquis	31.12	26.50	35.75
Penawawa	30.37	26.09	34.65
Currawa	29.91	25.49	34.32
Wakanz	29.30	25.07	33.53
Scarlet	28.96	24.71	33.22
Surprise	27.43	21.91	32.95
White Federation	25.32	20.92	29.73
Zak	24.15	19.56	28.73

genotype	%N	UCL	LCL
Zak	1.81	1.62	1.99
Currawa	1.72	1.54	1.90
Penawawa	1.70	1.53	1.87
White Federation	1.66	1.48	1.84
Idaed	1.55	1.37	1.72
Canus	1.54	1.37	1.71
Wakanz	1.50	1.33	1.67
Scarlet	1.45	1.28	1.63
Red Fife	1.38	1.21	1.55
White Marquis	1.31	1.12	1.50
Spinkcota	1.29	1.09	1.49
Onas	1.25	1.07	1.44
Westbred Express	1.24	1.05	1.43
Surprise	1.23	1.00	1.45
Pacific Bluestem	1.06	0.856	1.27
Pilcraw	1.05	0.875	1.22
Sonora	1.04	0.874	1.22
Bunyip	1.04	0.872	1.22
Arco	1.00	0.802	1.20

genotype	TN (g)	UCL	LCL
Idaed	0.493	0.449	0.536
Onas	0.472	0.425	0.518
Penawawa	0.466	0.422	0.509
Currawa	0.445	0.402	0.489
Canus	0.440	0.397	0.483
Pacific Bluestem	0.440	0.393	0.487
Westbred Express	0.416	0.370	0.463
Scarlet	0.413	0.370	0.457
Pilcraw	0.408	0.364	0.451
Arco	0.400	0.357	0.444
Wakanz	0.394	0.351	0.438
Bunyip	0.391	0.347	0.434
Zak	0.386	0.339	0.432
White Marquis	0.381	0.334	0.427
Sonora	0.377	0.334	0.420
Spinkcota	0.375	0.325	0.425
Red Fife	0.375	0.331	0.418
White Federation	0.370	0.327	0.413
Surprise	0.367	0.311	0.423

Table 4.7: Genotype LS mean values and 95% confidence intervals for individual means (Upper and Lower Confidence Limits) for grain weight, grain %N, grain TN, straw weight, straw %N and straw TN.

Grain at maturity

genotype	weight (g)	UCL	LCL
Wawawai	26.67	22.69	30.65
Onas	24.60	20.87	28.32
Penawawa	24.52	21.07	27.97
Bunyip	23.96	20.51	27.41
Zak	23.34	19.61	27.06
Pilcraw	22.58	19.13	26.03
White Federation	22.05	18.60	25.50
Canus	21.12	17.67	24.57
White Marquis	20.83	17.38	24.28
Idaed	19.82	16.37	23.27
Spinkcota	19.37	15.92	22.82
Alpowa	19.19	14.52	23.86
Currawa	19.19	15.47	22.92
Arco	19.11	15.12	23.09
Surprise	19.04	15.31	22.76
Wakanz	18.97	15.52	22.42
Scarlet	17.74	14.29	21.19
Westbred Express	16.71	13.26	20.16
Red Fife	16.33	12.88	19.78
Sonora	16.17	12.72	19.62
Pacific Bluestem	14.04	10.32	17.77

genotype	%N	UCL	LCL
Westbred Express	2.444	2.200	2.687
Pacific Bluestem	2.285	2.021	2.548
Sonora	2.282	2.038	2.526
Idaed	2.228	1.984	2.472
Currawa	2.195	1.932	2.459
Red Fife	2.071	1.827	2.314
White Federation	2.064	1.821	2.308
Scarlet	2.062	1.818	2.305
Spinkcota	2.061	1.817	2.305
White Marquis	2.019	1.775	2.262
Wakanz	1.879	1.635	2.122
Alpowa	1.879	1.548	2.209
Canus	1.861	1.618	2.105
Arco	1.850	1.569	2.132
Surprise	1.818	1.555	2.081
Zak	1.710	1.447	1.973
Bunyip	1.699	1.455	1.942
Onas	1.698	1.435	1.961
Penawawa	1.669	1.425	1.913
Pilcraw	1.594	1.350	1.837
Wawawai	1.536	1.255	1.818

genotype	TN (g)	UCL	LCL
White Federation	0.4493	0.4000	0.4986
Idaed	0.4358	0.3865	0.4851
White Marquis	0.4355	0.3862	0.4848
Currawa	0.4219	0.3686	0.4751
Wawawai	0.4115	0.3546	0.4685
Onas	0.4085	0.3552	0.4617
Bunyip	0.4070	0.3577	0.4563
Spinkcota	0.4048	0.3555	0.4541
Canus	0.4042	0.3549	0.4535
Zak	0.4023	0.3491	0.4556
Penawawa	0.3861	0.3368	0.4354
Westbred Express	0.3795	0.3302	0.4288
Surprise	0.3740	0.3207	0.4272
Scarlet	0.3641	0.3148	0.4134
Red Fife	0.3631	0.3138	0.4124
Pilcraw	0.3619	0.3126	0.4112
Arco	0.3494	0.2925	0.4064
Alpowa	0.3436	0.2769	0.4104
Sonora	0.3386	0.2893	0.3879
Wakanz	0.3352	0.2859	0.3845
Pacific Bluestem	0.2864	0.2332	0.3397

Straw at maturity

genotype	weight (g)	UCL	LCL
Pacific Bluestem	43.52	38.39	48.65
Pilcraw	40.69	35.94	45.45
Arco	37.73	32.25	43.22
Canus	36.93	32.18	41.68
Spinkcota	36.48	31.73	41.24
Bunyip	36.08	31.33	40.83
White Marquis	34.80	30.05	39.56
Onas	34.57	29.44	39.71
Surprise	32.96	27.83	38.09
Sonora	32.52	27.76	37.27
Penawawa	31.20	26.45	35.95
Zak	31.17	26.03	36.30
Wawawai	30.54	25.05	36.03
Red Fife	30.41	25.66	35.17
Wakanz	29.20	24.44	33.95
White Federation	27.94	23.19	32.69
Currawa	27.36	22.22	32.49
Idaed	27.08	22.32	31.83
Alpowa	26.87	20.43	33.30
Westbred Express	24.24	19.49	29.00
Scarlet	23.56	18.81	28.32

genotype	%N	UCL	LCL
Wakanz	0.5011	0.4303	0.5834
Alpowa	0.4993	0.4063	0.6136
Westbred Express	0.4950	0.4252	0.5764
Surprise	0.4090	0.3470	0.4821
Scarlet	0.4033	0.3464	0.4696
Zak	0.3861	0.3276	0.4551
Currawa	0.3532	0.2997	0.4163
White Federation	0.3506	0.3011	0.4082
Idaed	0.3450	0.2963	0.4017
Penawawa	0.3345	0.2873	0.3894
Pacific Bluestem	0.3315	0.2812	0.3907
Pilcraw	0.3231	0.2775	0.3763
Wawawai	0.3157	0.2648	0.3763
White Marquis	0.3136	0.2693	0.3651
Arco	0.3108	0.2607	0.3705
Sonora	0.3108	0.2669	0.3619
Spinkcota	0.3034	0.2605	0.3532
Onas	0.2997	0.2543	0.3532
Bunyip	0.2980	0.2559	0.3470
Red Fife	0.2957	0.2540	0.3443
Canus	0.2715	0.2332	0.3161

genotype	TN (g)	UCL	LCL
Wakanz	0.1528	0.1337	0.1719
Pacific Bluestem	0.1450	0.1243	0.1656
Surprise	0.1342	0.1136	0.1549
Pilcraw	0.1329	0.1138	0.1520
Alpowa	0.1311	0.1052	0.1569
Zak	0.1246	0.1040	0.1452
Westbred Express	0.1216	0.1025	0.1407
Arco	0.1176	0.09552	0.1396
Spinkcota	0.1150	0.09588	0.1341
Penawawa	0.1139	0.09479	0.1330
White Marquis	0.1112	0.09214	0.1303
Bunyip	0.1083	0.08923	0.1274
Sonora	0.1075	0.08839	0.1266
Onas	0.1073	0.08669	0.1279
Canus	0.1035	0.08444	0.1226
White Federation	0.1020	0.08288	0.1211
Currawa	0.1011	0.08045	0.1217
Scarlet	0.09687	0.07777	0.1160
Wawawai	0.09677	0.07472	0.1188
Red Fife	0.09622	0.07713	0.1153
Idaed	0.09599	0.07690	0.1151

Table 4.8: Genotype LS mean values and 95% confidence intervals for individual means (Upper and Lower Confidence Limits) for total AGB, HI, NHI, N uptake and N utilization efficiency.

genotype	HI	UCL	LCL
Wawawai	0.4683	0.4135	0.5231
White Federation	0.4429	0.3955	0.4904
Penawawa	0.4345	0.3870	0.4819
Alpowa	0.4340	0.3697	0.4982
Scarlet	0.4330	0.3856	0.4805
Zak	0.4272	0.3760	0.4785
Idaed	0.4228	0.3754	0.4703
Westbred Express	0.4165	0.3691	0.4640
Currawa	0.4119	0.3607	0.4632
Onas	0.4042	0.3530	0.4555
Bunyip	0.3964	0.3489	0.4438
Wakanz	0.3843	0.3369	0.4318
White Marquis	0.3762	0.3287	0.4236
Canus	0.3641	0.3167	0.4116
Pilcraw	0.3632	0.3157	0.4106
Surprise	0.3613	0.3101	0.4126
Spinkcota	0.3480	0.3006	0.3955
Red Fife	0.3467	0.2992	0.3941
Arco	0.3423	0.2876	0.3971
Sonora	0.3142	0.2667	0.3616
Pacific Bluestem	0.2374	0.1861	0.2886

genotype	NHI	UCL	LCL
White Federation	0.8146	0.7592	0.8701
Wawawai	0.8061	0.7421	0.8701
Currawa	0.8051	0.7452	0.8650
Idaed	0.8037	0.7483	0.8591
Canus	0.7877	0.7322	0.8431
White Marquis	0.7845	0.7291	0.8399
Onas	0.7838	0.7239	0.8436
Bunyip	0.7826	0.7271	0.8380
Spinkcota	0.7784	0.7230	0.8339
Penawawa	0.7701	0.7147	0.8256
Scarlet	0.7697	0.7142	0.8251
Red Fife	0.7686	0.7132	0.8240
Westbred Express	0.7615	0.7061	0.8170
Zak	0.7556	0.6957	0.8155
Sonora	0.7340	0.6785	0.7894
Arco	0.7338	0.6698	0.7978
Alpowa	0.7249	0.6499	0.8000
Pilcraw	0.7038	0.6484	0.7593
Surprise	0.6810	0.6211	0.7409
Pacific Bluestem	0.6610	0.6012	0.7209
Wakanz	0.6552	0.5998	0.7106

genotype	N ut	UCL	LCL
Wawawai	54.48	47.64	61.31
Penawawa	52.38	46.46	58.30
Onas	48.98	42.58	55.37
Bunyip	48.05	42.14	53.97
Zak	47.30	40.91	53.69
Pilcraw	47.27	41.35	53.19
Canus	45.07	39.15	50.99
Arco	44.10	37.26	50.94
White Federation	43.07	37.15	48.99
Scarlet	42.40	36.48	48.31
Alpowa	42.33	34.32	50.35
Idaed	40.73	34.81	46.65
White Marquis	40.44	34.52	46.36
Red Fife	39.15	33.23	45.07
Spinkcota	39.11	33.19	45.02
Currawa	39.06	32.67	45.45
Surprise	39.05	32.66	45.45
Wakanz	37.80	31.88	43.72
Sonora	37.42	31.50	43.34
Westbred Express	35.54	29.62	41.46
Pacific Bluestem	33.56	27.16	39.95

genotype	AGB (g)	UCL	LCL
Pilcraw	63.27	56.81	69.72
Bunyip	60.04	53.59	66.50
Onas	59.17	52.20	66.14
Canus	58.05	51.59	64.50
Pacific Bluestem	57.56	50.59	64.53
Wawawai	57.21	49.76	64.67
Arco	56.84	49.38	64.29
Spinkcota	55.85	49.40	62.31
Penawawa	55.72	49.26	62.17
White Marquis	55.63	49.18	62.09
Zak	54.50	47.53	61.47
Surprise	52.00	45.02	58.97
White Federation	49.99	43.54	56.45
Sonora	48.68	42.23	55.14
Wakanz	48.17	41.71	54.62
Idaed	46.89	40.44	53.35
Red Fife	46.74	40.29	53.20
Currawa	46.55	39.58	53.52
Alpowa	46.06	37.32	54.80
Scarlet	41.30	34.84	47.76
Westbred Express	40.95	34.50	47.41

genotype	N up (g)	UCL	LCL
White Federation	0.5513	0.5045	0.5980
White Marquis	0.5467	0.5000	0.5935
Idaed	0.5318	0.4850	0.5785
Zak	0.5269	0.4765	0.5774
Currawa	0.5229	0.4724	0.5734
Spinkcota	0.5198	0.4731	0.5665
Onas	0.5158	0.4653	0.5662
Bunyip	0.5153	0.4686	0.5621
Wawawai	0.5083	0.4543	0.5623
Surprise	0.5082	0.4577	0.5587
Canus	0.5078	0.4610	0.5545
Westbred Express	0.5011	0.4543	0.5478
Penawawa	0.4999	0.4532	0.5467
Pilcraw	0.4948	0.4480	0.5415
Wakanz	0.4880	0.4413	0.5348
Alpowa	0.4747	0.4114	0.5380
Arco	0.4670	0.4130	0.5210
Scarlet	0.4609	0.4142	0.5077
Red Fife	0.4593	0.4126	0.5060
Sonora	0.4461	0.3994	0.4928
Pacific Bluestem	0.4314	0.3809	0.4818

4.3.2 Chromosome addition lines

SPAD was significant as a covariate for %N at the 6leaf stage. DAP was significant for %N and AGB at the anthesis stage and for grain %N, straw %N and straw TN at maturity (Table 4.4 and Table 4.5). Slopes were generally small and positive, except for %N at anthesis, which was negative. For all other measured variables, the covariate was dropped and an ANOVA model was used.

The environmental component of variation was important for all measured variables except for %N at the 6leaf stage and for grain weight, straw TN, AGB, N utilization and NHI at maturity. Interaction between the genotype and cycle was apparent for most measured variables. An interaction between the SPAD meter reading and the genotype was present for 6leaf %N (Table 4.4). Genotypic main effects were significant for all variables at the 6leaf stage, none of the variables at anthesis and all but straw %N, straw TN, AGB, and N uptake at maturity.

LS mean values for 6leaf and anthesis are given in Table 4.3.2. At the 6leaf stage, AgCS had significantly greater AGB than all genotypes except CS+4E and greater TN than all genotypes except CS and CS+4E. For %N, CS+7E had greater %N than CS, CS+3E and *Th. elongatum*, and CS had significantly lower %N than CS+1E and CS+6E. *Th. elongatum* had lower %N than all the chromosome addition lines but was not significantly different than CS or AgCS. At anthesis, there were few significant differences among genotypes. CS+1E had greater TN than CS+5E,

Table 4.9: LS mean values and 95% confidence intervals for individual means (Upper and Lower Confidence Limits) for chromosome addition lines and parental genotypes at the 6leaf and anthesis stages for AGB, %N and TN.

6leaf

genotype	weight (g)	LCL	UCL
AgCs	2.00	1.631	2.373
Cs+4E	1.21	0.839	1.581
Cs	1.05	0.681	1.423
Cs+6E	1.01	0.643	1.385
Cs+5E	0.98	0.606	1.348
Cs+3E	0.89	0.521	1.263
Cs+1E	0.86	0.461	1.262
Cs+7E	0.83	0.463	1.205
Elongatum	0.76	0.389	1.131
Cs+2E	0.49	0.000	0.993

genotype	%N	LCL	UCL
Cs+7E	6.143	5.903	6.383
Cs+2E	6.064	5.694	6.435
Cs+1E	5.996	5.797	6.195
Cs+6E	5.899	5.761	6.038
Cs+5E	5.882	5.653	6.110
Cs+4E	5.742	5.618	5.866
Cs+3E	5.601	5.448	5.753
Cs	5.511	5.345	5.677
Elongatum	5.265	5.141	5.389
AgCs	5.204	4.679	5.730

genotype	TN (g)	LCL	UCL
AgCs	0.1020	0.0833	0.1210
Cs+4E	0.0694	0.0505	0.0882
Cs	0.0592	0.0403	0.0780
Cs+6E	0.0564	0.0376	0.0753
Cs+5E	0.0560	0.0372	0.0749
Cs+3E	0.0518	0.0330	0.0707
Cs+7E	0.0481	0.0293	0.0670
Cs+1E	0.0439	0.0235	0.0642
Elongatum	0.0360	0.0172	0.0549
Cs+2E	0.0271	0.0016	0.0526

Anthesis

genotype	weight (g)	LCL	UCL
Cs+1E	38.81	34.30	43.32
Cs	37.91	33.21	42.61
Cs+7E	36.75	32.42	41.08
AgCs	34.21	28.53	39.88
Cs+3E	34.03	28.68	39.38
Cs+6E	33.85	29.39	38.31
Cs+4E	33.71	28.90	38.52
Cs+5E	33.28	28.38	38.18
Elongatum	29.65	24.30	35.01
Cs+2E	25.36	20.20	30.52

genotype	%N	LCL	UCL
Cs+2E	1.930	1.730	2.131
Cs+1E	1.700	1.525	1.876
Cs+3E	1.640	1.432	1.848
Cs+4E	1.621	1.434	1.808
Cs+6E	1.564	1.391	1.737
Cs+7E	1.543	1.375	1.711
Elongatum	1.513	1.305	1.721
AgCs	1.504	1.283	1.725
Cs+5E	1.473	1.283	1.663
Cs	1.392	1.209	1.575

genotype	TN (g)	LCL	UCL
Cs+1E	0.5847	0.5213	0.6481
Cs+4E	0.5285	0.4650	0.5919
Cs+7E	0.5242	0.4607	0.5876
Cs	0.5218	0.4583	0.5852
Cs+6E	0.4796	0.4162	0.5430
Cs+2E	0.4790	0.4105	0.5475
AgCs	0.4645	0.4010	0.5279
Cs+5E	0.4303	0.3669	0.4938
Elongatum	0.4272	0.3638	0.4906
Cs+3E	0.4269	0.3635	0.4903

CS+3E and *Th. elongatum*. CS+2E had greater %N than *Th. elongatum*, AgCS and CS. CS+1E had significantly greater AGB than CS+2E and *Th. elongatum*.

Table 4.10 gives LS mean values for the chromosome addition lines and parental genotypes at maturity. CS+7E had greater grain %N than all genotypes except CS+5E and CS+6E. CS+5E had higher %N than CS and AgCS, and CS+6E and CS+1E had higher grain %N than AgCS. In terms of total grain N, CS+3E was greater than CS+5E, CS+6E, CS+4E, AgCS and *Th. elongatum*. CS was greater than AgCS and *Th. elongatum*. CS+2E, CS+7E, and CS+1E were all greater than AgCS. *Th. elongatum* had greater straw weight than Cs+1E, Cs+7E, Cs+2E, Cs+3E, and Cs+5E and AgCS had greater straw weight than CS+5E. For straw %N, there was only one significant difference, with CS+5E greater than AgCS.

Table 4.10: LS mean values and 95% confidence intervals for individual means (Upper and Lower Confidence Limits) for chromosome addition lines and parental genotypes at maturity for grain weight, grain %N, grain TN, straw weight, straw %N and straw TN.

Grain at maturity

genotype	weight (g)	LCL	UCL
Cs	16.30	12.86	19.75
Cs+3E	16.01	12.57	19.45
Cs+2E	12.04	7.38	16.70
Cs+4E	9.68	6.24	13.13
Cs+1E	9.37	5.93	12.81
Cs+5E	8.13	4.69	11.57
Cs+6E	7.57	4.13	11.01
Cs+7E	7.42	3.44	11.39
AgCs	7.24	3.80	10.69
Elongatum	1.46	0.00	4.90

genotype	%N	LCL	UCL
Cs+7E	4.327	3.887	4.766
Cs+5E	3.610	3.145	4.074
Cs+6E	3.552	3.157	3.947
Cs+1E	3.479	3.099	3.860
Cs+4E	3.235	2.893	3.577
Cs+3E	3.146	2.728	3.564
Cs	2.806	2.377	3.235
Cs+2E	2.477	1.997	2.956
AgCs	2.070	1.489	2.651
Elongatum	1.361	0.030	2.692

genotype	TN (g)	LCL	UCL
Cs+3E	0.4381	0.3587	0.5176
Cs	0.3854	0.3059	0.4649
Cs+2E	0.3357	0.2281	0.4433
Cs+7E	0.3127	0.2209	0.4044
Cs+1E	0.2718	0.1923	0.3513
Cs+5E	0.2414	0.1619	0.3209
Cs+6E	0.2405	0.1610	0.3199
Cs+4E	0.2259	0.1464	0.3053
AgCs	0.1889	0.1094	0.2684
Elongatum	0.0603	0.0000	0.1461

Straw at maturity

genotype	weight (g)	LCL	UCL
Elongatum	52.87	46.06	59.68
AgCs	47.06	40.26	53.87
Cs+4E	46.53	39.72	53.34
Cs+6E	39.95	33.14	46.76
Cs	39.27	32.47	46.08
Cs+1E	34.85	28.05	41.66
Cs+7E	34.36	26.50	42.21
Cs+2E	33.55	24.33	42.76
Cs+3E	33.02	26.21	39.83
Cs+5E	31.21	24.40	38.02

genotype	%N	UCL	LCL
Cs+5E	0.6362	0.5098	0.7940
Cs+3E	0.5731	0.4648	0.7068
Cs+1E	0.5430	0.4444	0.6635
Cs+6E	0.5365	0.4376	0.6578
Cs	0.5135	0.4153	0.6350
Cs+4E	0.4745	0.3917	0.5747
Cs+7E	0.4601	0.3650	0.5799
Cs+2E	0.4282	0.3291	0.5572
AgCs	0.3131	0.2429	0.4034
Elongatum	0.2851	0.1844	0.4407

genotype	TN (g)	LCL	UCL
Cs+4E	0.2188	0.1776	0.2696
Cs+6E	0.2177	0.1744	0.2719
Cs	0.2126	0.1687	0.2680
Cs+5E	0.1975	0.1552	0.2515
Cs+3E	0.1940	0.1544	0.2438
Cs+1E	0.1939	0.1559	0.2413
Cs+7E	0.1642	0.1276	0.2114
Cs+2E	0.1351	0.1014	0.1799
AgCs	0.1237	0.0938	0.1630
Elongatum	0.1005	0.0625	0.1616

Finally, for grain weight, CS was greater than CS+6E, CS+7E, AgCS, and *Th. elongatum*. *Th. elongatum* was also less than CS+3E, CS+2E, and CS+4E, and CS+3E was greater than CS+6E.

Th. elongatum had a lower HI than all but CS+4E and AgCS. CS+3E was greater than these three, CS+6E and CS+7E. CS was also significantly greater than AgCS. There were fewer significant differences for NHI, with *Th. elongatum* significantly less than all but CS+4E; and CS+3E significantly greater than CS+4E. For N uptake CS and CS+3E were greater than AgCS and *Th. elongatum*. CS was greater than CS+4E, CS+6E, CS+7E, and *Th. elongatum* in terms of N utilization efficiency, and *Th. elongatum* was significantly less efficient than all but CS+4E,CS+6E and CS+7E. There were no significant differences for AGB or straw

Table 4.11: LS mean values and 95% confidence intervals for individual means (Upper and Lower Confidence Limits) for chromosome addition lines and parental genotypes at maturity for total aboveground plant AGB, HI, NHI, N up and N utilization efficiency.

genotype	HI	LCL	UCL
Cs+3E	0.335	0.278	0.392
Cs	0.289	0.233	0.346
Cs+2E	0.260	0.184	0.337
Cs+5E	0.220	0.163	0.277
Cs+1E	0.204	0.147	0.261
Cs+7E	0.178	0.112	0.243
Cs+6E	0.168	0.111	0.225
Cs+4E	0.161	0.104	0.218
AgCs	0.138	0.081	0.195
Elongatum	0.029	0.000	0.086

genotype	NHI	LCL	UCL
Cs+3E	0.7111	0.6043	0.8180
Cs+2E	0.6701	0.5255	0.8148
Cs	0.6571	0.5503	0.7639
Cs+7E	0.6152	0.4919	0.7386
Cs+1E	0.5783	0.4715	0.6852
Cs+5E	0.5737	0.4669	0.6805
Cs+6E	0.5205	0.4136	0.6273
AgCs	0.5104	0.4036	0.6172
Cs+4E	0.4117	0.3049	0.5185
Elongatum	0.1772	0.0618	0.2925

genotype	N ut	LCL	UCL
Cs	29.6	24.2	35.0
Cs+3E	27.5	22.1	32.8
Cs+2E	25.1	17.8	32.4
Cs+5E	19.6	14.2	25.0
AgCs	19.6	14.2	25.0
Cs+1E	19.4	14.1	24.8
Cs+4E	16.9	11.5	22.3
Cs+6E	16.3	10.9	21.7
Cs+7E	15.6	9.3	21.8
Elongatum	5.5	0.0	11.3

genotype	AGB (g)	LCL	UCL
Cs+4E	56.21	48.77	63.66
Cs	55.58	48.13	63.02
Elongatum	54.33	46.88	61.77
AgCs	54.31	46.86	61.75
Cs+3E	49.03	41.59	56.47
Cs+6E	47.52	40.08	54.96
Cs+2E	45.59	35.51	55.67
Cs+1E	44.22	36.78	51.67
Cs+7E	41.77	33.18	50.37
Cs+5E	39.34	31.90	46.79

genotype	N up (g)	LCL	UCL
Cs+3E	0.6070	0.5313	0.6827
Cs	0.5873	0.5116	0.6630
Cs+2E	0.5013	0.3988	0.6038
Cs+7E	0.4621	0.3746	0.5495
Cs+4E	0.4553	0.3796	0.5310
Cs+1E	0.4498	0.3741	0.5255
Cs+6E	0.4357	0.3600	0.5114
Cs+5E	0.4135	0.3378	0.4893
AgCs	0.3869	0.3112	0.4626
Elongatum	0.3632	0.2814	0.4450

TN and only one significant difference for straw %N, with CS+5E being greater than AgCS.

4.3.3 Perennial lines

The covariate DAP was significant for %N and AGB at anthesis and straw TN and straw weight at maturity. SPAD was significant for %N at anthesis. For all other variables an ANOVA model was used. The slopes of the covariates were small, with DAP being negatively correlated with %N at anthesis and positively correlated with anthesis AGB, straw TN and straw weight. SPAD was positively correlated with %N at anthesis.

The environmental component of variation was significant for all variables

except AGB at anthesis, and grain %N, straw TN and N utilization efficiency at maturity. Interactions between cycle and genotype were more prevalent than genotypic main effects (Table 4.4 and Table 4.5). Genotypic effects were significant for grain %N, straw %N, straw weight, and AGB at maturity.

Comparisons among individual genotypes showed no significant differences at the 6leaf stage. At anthesis, 03JP039 had significantly less AGB than 03JP026, and 03JP026 had significantly lower % N than all other genotypes except 03JP022 and SS259 (Table 4.12 gives LS mean values for perennial lines a 6leaf and anthesis).

At maturity, 03JP039 had significantly less AGB and higher grain %N than 03JP004, 03JP019, 03JP026, and PI550713 and higher grain %N than SS259 and 03JP016. 03JP039 also had higher %N than 03JP016 and 03JP019. There were not many significant differences for grain weight, with 03JP016 significantly greater than 03JP036 and 03JP039, and PI550713 also greater than 03JP039. For straw %N, 03JP039 had greater concentrations than 03JP004, 03JP016, SS259 and 03JP019. Also for straw %N, 03JP022, 03JP036 and 03JP026 had greater concentrations than SS259 and 03JP019. For straw TN, 03JP039 had higher levels than 03JP019, SS259 and 03JP016. There were no differences for grain TN, HI, or N up. For N utilization efficiency, 03JP019 was more efficient than 03JP036 and 03JP039. For NHI, 03JP016 was greater than 03JP026. For straw weight, 03JP019 was significantly greater than 3JP039, and 03JP016 was significantly less than both 03JP026 and 03JP022 (LS mean values given in Table 4.13 and Table 4.14).

Table 4.12: LS means and 95% confidence intervals for individual means (Upper and Lower Confidence Limits) for perennial lines at 6leaf and anthesis stages for AGB, %N, and TN.

6leaf

genotype	weight (g)	LCL	UCL
SS259	0.79	0.61	0.97
03JP019	0.73	0.55	0.91
PI550713	0.72	0.54	0.90
03JP004	0.64	0.46	0.82
03JP016	0.63	0.45	0.81
03JP036	0.62	0.44	0.80
03JP039	0.54	0.36	0.72
03JP026	0.50	0.31	0.68
03JP022	0.41	0.23	0.59

genotype	%N	LCL	UCL
03JP016	5.83	5.61	6.05
03JP022	5.67	5.45	5.89
03JP004	5.65	5.43	5.87
03JP026	5.63	5.41	5.85
03JP039	5.52	5.31	5.74
03JP019	5.47	5.25	5.69
SS259	5.38	5.16	5.60
03JP036	5.37	5.15	5.59
PI550713	5.36	5.14	5.58

genotype	TN (g)	LCL	UCL
SS259	0.043	0.033	0.054
03JP019	0.041	0.031	0.051
PI550713	0.040	0.030	0.050
03JP016	0.036	0.026	0.046
03JP004	0.036	0.026	0.046
03JP036	0.034	0.024	0.044
03JP039	0.031	0.021	0.041
03JP026	0.029	0.019	0.039
03JP022	0.023	0.013	0.033

Anthesis

genotype	weight (g)	LCL	UCL
03JP026	40.63	36.44	44.82
03JP016	38.05	33.92	42.19
SS259	37.59	33.24	41.93
03JP004	36.77	32.60	40.94
03JP036	35.81	30.16	41.47
03JP022	35.75	31.63	39.87
03JP019	33.18	28.48	37.87
PI550713	33.07	28.95	37.19
03JP039	30.23	25.82	34.63

genotype	%N	LCL	UCL
03JP039	1.658	1.473	1.867
PI550713	1.450	1.281	1.642
03JP019	1.412	1.247	1.598
03JP036	1.367	1.179	1.585
03JP016	1.357	1.219	1.512
03JP004	1.342	1.204	1.495
03JP022	1.285	1.152	1.433
SS259	1.269	1.132	1.423
03JP026	1.019	0.9139	1.136

genotype	TN (g)	LCL	UCL
03JP016	0.5393	0.4829	0.5957
03JP004	0.5279	0.4714	0.5843
03JP036	0.5253	0.4643	0.5862
03JP039	0.5016	0.4451	0.5580
SS259	0.4995	0.4431	0.5559
03JP019	0.4796	0.4186	0.5406
03JP026	0.4661	0.4096	0.5225
03JP022	0.4619	0.4055	0.5184
PI550713	0.4325	0.3761	0.4890

Table 4.13: LS means and 95% confidence intervals for individual means (Upper and Lower Confidence Limits) for perennial lines at maturity for grain weight, grain %N, grain TN, straw weight, straw %N, and straw TN.

Grain at maturity

genotype	weight (g)	LCL	UCL
03JP016	18.04	14.76	21.33
PI550713	16.90	13.61	20.18
03JP004	16.51	12.97	20.06
03JP019	16.34	13.06	19.62
SS259	15.39	12.11	18.68
03JP026	12.76	9.47	16.04
03JP022	12.38	9.10	15.67
03JP036	10.36	7.07	13.64
03JP039	9.08	5.80	12.37

genotype	%N	LCL	UCL
03JP039	3.993	3.563	4.424
03JP036	3.504	3.074	3.934
03JP022	3.061	2.631	3.491
03JP004	2.793	2.328	3.257
SS259	2.744	2.314	3.174
PI550713	2.618	2.188	3.048
03JP026	2.559	2.129	2.989
03JP016	2.465	2.035	2.896
03JP019	2.392	1.962	2.823

genotype	TN (g)	LCL	UCL
03JP004	0.4519	0.3548	0.5490
03JP016	0.4399	0.3500	0.5298
PI550713	0.4357	0.3458	0.5256
SS259	0.4013	0.3114	0.4912
03JP019	0.3724	0.2824	0.4623
03JP022	0.3709	0.2810	0.4608
03JP039	0.3473	0.2574	0.4372
03JP036	0.3349	0.2450	0.4248
03JP026	0.3250	0.2351	0.4149

Straw at maturity

genotype	weight (g)	LCL	UCL
03JP019	51.43	45.11	57.75
03JP026	49.08	42.92	55.23
PI550713	47.43	41.66	53.20
03JP022	45.67	39.75	51.59
SS259	45.43	39.39	51.48
03JP004	45.42	38.24	52.59
03JP016	42.95	37.09	48.81
03JP036	38.14	32.25	44.02
03JP039	35.50	29.76	41.24

genotype	%N	LCL	UCL
03JP039	0.8256	0.6582	1.0356
03JP022	0.6120	0.4879	0.7677
03JP036	0.6003	0.4786	0.7530
03JP026	0.5875	0.4684	0.7369
PI550713	0.4903	0.3909	0.6150
03JP004	0.4089	0.3202	0.5223
03JP016	0.3684	0.2937	0.4621
SS259	0.3470	0.2766	0.4352
03JP019	0.3425	0.2731	0.4296

genotype	TN (g)	LCL	UCL
03JP039	0.2822	0.2318	0.3435
03JP026	0.2621	0.2122	0.3236
03JP022	0.2620	0.2139	0.3210
PI550713	0.2303	0.1890	0.2806
03JP036	0.2171	0.1774	0.2656
03JP019	0.1789	0.1440	0.2221
03JP004	0.1687	0.1319	0.2158
SS259	0.1595	0.1297	0.1963
03JP016	0.1584	0.1296	0.1936

Table 4.14: LS means and 95% confidence intervals for individual means (Upper and Lower Confidence Limits) for perennial lines at maturity for total AGB, HI, NHI, N uptake, and N utilization efficiency

genotype	HI	LCL	UCL
03JP016	0.2791	0.2335	0.3247
PI550713	0.2508	0.2052	0.2964
03JP019	0.2416	0.1960	0.2871
SS259	0.2326	0.1871	0.2782
03JP004	0.2266	0.1774	0.2758
03JP036	0.1950	0.1494	0.2405
03JP022	0.1924	0.1468	0.2379
03JP039	0.1859	0.1404	0.2315
03JP026	0.1807	0.1351	0.2262

genotype	NHI	STD ERR	STD ERR
03JP016	0.7007	0.5929	0.8085
SS259	0.6615	0.5537	0.7693
03JP004	0.6253	0.5089	0.7418
PI550713	0.6139	0.5061	0.7217
03JP019	0.6072	0.4994	0.7149
03JP022	0.5041	0.3963	0.6119
03JP036	0.4852	0.3774	0.5930
03JP039	0.4846	0.3768	0.5924
03JP026	0.4361	0.3283	0.5439

genotype	N ut	LCL	UCL
03JP019	36.92	27.81	46.02
03JP016	29.12	20.02	38.22
SS259	25.69	16.59	34.79
PI550713	24.63	15.53	33.73
03JP004	23.61	13.78	33.44
03JP022	18.34	9.23	27.44
03JP026	17.92	8.82	27.02
03JP036	15.48	6.38	24.58
03JP039	13.04	3.94	22.14

genotype	AGB (g)	LCL	UCL
03JP004	65.86	57.60	74.12
03JP019	64.84	51.76	67.06
03JP026	64.36	57.19	72.49
PI550713	63.15	52.22	67.52
03JP022	59.87	56.71	72.01
03JP016	59.41	42.53	57.83
SS259	58.60	35.94	51.24
03JP036	50.18	55.50	70.80
03JP039	43.59	50.95	66.25

genotype	N up (g)	LCL	UCL
03JP022	0.6868	0.6082	0.7653
03JP039	0.6693	0.5907	0.7478
PI550713	0.6667	0.5881	0.7452
03JP004	0.6615	0.5766	0.7464
03JP026	0.6366	0.5580	0.7151
03JP016	0.6035	0.5249	0.6820
03JP036	0.5966	0.5181	0.6752
SS259	0.5565	0.4779	0.6350
03JP019	0.5483	0.4698	0.6269

4.4 Disscussion

4.4.1 Annual varieties

Historic varieties produced significantly more AGB but had lower tissue N concentrations than modern varieties at all stages of growth. At maturity, the higher AGB was due to higher straw weight as grain weight was not significantly different between the two categories. The higher HI of modern varieties is often associated with higher grain weight (Donmez et al., 2001; Sinclair, 1998; Woodruff, 1972). However, in this experiment using organic fertilizer, the historic cultivars did not differ significantly from modern cultivars in terms of grain weight, grain %N, grain TN, straw TN, N uptake, N utilization efficiency or NHI. Under conditions where N is supplied organically, historic genotypes may therefore possess favorable traits for

taking up and using N to produce AGB, grain and grain protein. In a field study of wheat varieties, Singh and Arora (2001) found that tall genotypes had significantly higher N uptake than dwarf types at two N levels tested.

Interestingly, there were no significant differences for total N uptake among the genotypes tested in this study. This is similar to the results of Dubois and Fossati (1981) in a study of winter wheat genotypes, where there were no differences in total plant N. Desai and Bhatia (1978) found contrasting results, where total plant N was positively correlated to total AGB, grain yield, and grain TN. The authors concluded that plant capacity for N uptake was the most important factor in increasing both grain yield and grain TN. However, our study showed no significant differences for N uptake or NHI, but significant differences among genotypes for AGB, grain weight and grain TN, therefore there are likely other factors involved in both increasing yield and grain TN than simple uptake and partitioning. Dubois and Fossati (1981) also concluded that high plant N uptake by itself was not critical to obtaining high yield, grain %N and grain TN.

Across all categories, genotypes had accumulated an average of 83% of the plant TN at maturity by anthesis. This is in line with previous studies, as Cox et al. (1985) found that about 82-83% of N in grain was already in the plant at anthesis. The NHI range of 66-81% in this study corresponds to other values published in the literature as more than 75% of total N is partitioned into the grain on average (Dhugga and Waines, 1989).

There were significant differences among genotypes within each category for many of the measured variables, and this information could be used to chose parents that combine useful traits for efficient N use. In general, those genotypes with high AGB had low %N and vice versa. At maturity, although there were significant differences between pairs of genotypes, the best performing genotypes and worst performing genotypes for each trait included historic and modern varieties. The range of variation for these traits means that lines with favorable combinations could be crossed and then selection could be carried out under organic fertility management to create new varieties with specific adaptation to these conditions.

4.4.2 Addition lines

The addition of perennial chromosomes to Chinese Spring wheat had a differential effect on traits related to N use at 6leaf, and on grain traits, straw weight, N utilization efficiency, NHI and HI at maturity. As environmental and interaction terms are also significant, it appears that the effects of these chromosomes may differ according to environmental conditions.

A comparison of LS mean values showed some interesting trends, particularly for CS+4E, which has a perennial growth habit (Lammer et al., 2004). This line tended to group with *Th. elongatum* and the amphiploid AgCS. If the N dynamics of this line are more similar to the wild perennial parent than the annual spring wheat

parent, it could have significant implications for the breeding program. However, there were many cases when CS+4E was not significantly different than the other chromosome addition lines, and none of the chromosome addition lines had significantly lower yield than annual CS. The chromosome addition lines CS+2E, CS+3E and CS+7E all had good performance in terms of N concentration traits, and these chromosomes could be important for plant N uptake.

The addition lines tended to have the widest range for measured variables, compared to annual and perennial lines. This was particularly true at maturity for grain and straw traits, because of the low yield and N status of *Th. elongatum*. While elongatum had the greatest straw weight and high total AGB production compared to the other lines in the addition experiment, it had very low grain weight, grain and straw %N and grain and straw TN. This led to very low values for HI, NHI and N utilization efficiency. Other lines in the addition experiment were comparable to perennial lines and the lower range of annual genotypes for grain weight and TN, and tended to have higher grain %N and straw TN than annual lines. HI, NHI and N utilization efficiency values for these lines were also comparable to the perennials and to the lower range of annual genotypes.

4.4.3 Perennial lines

The effect of perennial genotype was nonsignificant for measured variables during the vegetative growth stages. Genotypic effects were apparent at maturity for grain and straw %N, straw weight and AGB, but not for other traits. However, interaction effects between genotype and cycle were significant for several traits where there were no genotypic main effects. Even when genotype effects were not significant in the ANOVA, individual genotypes can be identified with significantly better performance for those traits. The best genotypes for each measured variable related to NUE could be crossed and selected to improve these traits.

In comparison to annual genotypes and chromosome addition lines, the perennial lines had similar %N at 6leaf and anthesis. While the perennial AGB was lower at 6leaf it was very similar to the other two experiments at anthesis. TN values at these stages followed the same pattern as AGB values. At maturity, perennial lines tended to have lower grain weight than the higher yielding annuals, but had grain weights overlapping the lower end of the annual range. Grain %N tended to be higher in the perennials and grain TN values were similar. Straw weights were also similar, with a wider range of %N in the perennials and generally greater TN values in perennial lines. The HI was lower in perennials, but overlapped the lower range of the annual genotypes. NHI showed the same pattern. Total AGB production was similar between perennials and annuals and N up values tended to be higher in the perennial genotypes.

4.4.4 Relationship of SPAD and DAP to plant N status

SPAD meter readings were correlated with %N at the 6leaf stage in chromosome addition lines and at the anthesis stage in perennial genotypes. This confirms findings in previous studies that chlorophyll content is positively correlated with tissue N concentration (Vidal et al., 1999; Gáborčík, 2003; Giunta et al., 2002). The SPAD values were not significantly associated with TN or AGB at the 6-leaf or anthesis stage in any of the greenhouse experiments. In addition, the slope of the regression line between SPAD and %N was small, only 0.04 at 6leaf in the experiment with chromosome addition lines and 0.02 at the anthesis stage in the experiment with perennial genotypes.

DAP was significant more frequently, but the slopes were also quite small. At the 6-leaf stage, DAP had a small positive slope for all three traits measured in the chromosome addition lines, meaning that plants simultaneously increased tissue N concentration and AGB. DAP was most important for AGB at anthesis, with a slope of 0.7023 for the chromosome addition lines experiment and 0.4512 for the perennial line experiment. For %N at anthesis in both chromosome addition line experiment and perennial line experiment, the slope of dap was negative, -0.0268 and -0.0082, respectively. This is what would be expected if genotypes reaching anthesis later after planting accumulated more AGB but not necessarily more N. DAP was insignificant for TN at anthesis for the chromosome addition and perennial lines, so it appears that there is a trade-off at this stage between N concentration and AGB. For

the chromosome addition and perennial lines, the timing and duration of senescence was much more variable than in annual lines. DAP was significant for grain %N, straw %N and total straw N and for straw TN and straw weight in perennials.

In the annual experiment, the covariate DAP was positively correlated with AGB traits and negatively correlated with %N traits in the vegetative stages. It appears that the lines that take longer to reach the six leaf or anthesis stage have greater AGB at these physiological points. N concentration dropped as plants grew, possibly as a result of dilution. However, TN continued to increase, as the slope of DAP is positive for TN at the 6leaf stage. DAP was not significant for plant TN, either in the vegetative stages or at maturity, so it seems that N uptake is not dependent on the length of time it takes to reach certain physiological stages. At maturity, DAP was not important for any traits, most likely because annual genotypes senesced quickly and completely without much variation in maturity date. The date of release was also not significant, which is not unexpected given that the historic and modern categories were not significantly different for many of the measured variables. Interestingly, the market class was also not significant, meaning that the N utilization of these plants seems to be independent of whether they are hard or soft wheat.

4.5 Conclusions

From this study, it is apparent that there is significant genetic variation for traits related to plant N uptake and partitioning when grown with organic N sources in the controlled environment of the greenhouse. For annual spring wheat, historic varieties had greater plant AGB but were not significantly different from modern varieties for grain weight, grain %N, grain TN, straw TN, N uptake, N utilization efficiency or NHI. This differs from many field experiments and shows that in the absence of disease, insect and drought stress, historic genotypes have favorable traits for efficient N use that could be useful when incorporated into varieties with adaptation to current field conditions.

Significant genetic differences were also found among the chromosome addition lines and among perennial genotypes. Perennial and addition lines tended to have greater variation among the lines and some lines overlapped with annual genotypes for traits of relevance to NUE. In particular, perennial genotypes were able to produce adequate AGB and had similar or greater N uptake than annual lines. The values for the perennial lines tended to be much closer to the annual means than to the values for *Th. elongatum*, their wild wheat parent. Improvements in the HI and NHI of these lines will be necessary for them to be viable agronomic crops, but they are already capable of assimilating enough TN and AGB to be comparable to existing annual genotypes under organic conditions. This information can be used in

developing perennial wheat lines adapted to low input and organic conditions.

Acknowledgments

The authors would like to thank Steven Lyon, Kerry Balow, Meg Gollnick, Kevin Murphy, Julia Piaskowski, Matt Arterburn and Amy Hetrick for extensive help and advice with this experiment. Margaret Davies and Ron Bolton provided technical expertise in the analysis of soil and plant samples. Richard Alldredge was of great help in the statistical analysis. This work was funded by the National Science Foundation under a Graduate Research Fellowship and by a Graduate Research Fellowship of The Land Institute, Salina, KS. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation or the Land Institute.

Bibliography

Cox M., Qualset C.O., Rains D.W., 1985. Genetic variation for nitrogen assimilation and translocation in wheat II: Nitrogen assimilation in relation to grain yield and protein. Crop Science 25, 435–440.

Dawson J.C., Huggins D.R., Jones S.S., 2007. Characterizing nitrogen use efficiency

in natural and agricultural ecosystems to improve the performance of low input and organic agricultural systems. Field Crops Research Submitted August 2007.

Desai R.M., Bhatia C., 1978. Nitrogen uptake and nitrogen harvest index in durum wheat cultivars varying in their grain protein concentration. Euphytica 27, 561–566.

Dhugga K.S., Waines J.G., 1989. Analysis of nitrogen accumulation and use in bread and durum wheat. Crop Science 29, 1232–1239.

Donmez E., Sears R.G., Shroyer J.P., Paulsen G.M., 2001. Genetic gain in yield attributes of winter wheat in the great plains. Crop Science 41, 1412–1419.

Dubois J.B., Fossati A., 1981. Influence of nitrogen uptake and nitrogen partitioning efficiency on grain yield and grain protein concentration of twelve winter wheat genotypes (*Triticum aestivum* L.). Zeitschrift fur Pflansensuchtung=Journal of Plant Breeding 86, 41–49.

Dvorak J., Knott D.R., 1974. Disomic and ditelosomic additions of diploid *Agropyron elongatum* chromosomes to *Triticum aestivum*. Canadian Journal of Genetics and Cytology 16, 399–417.

Foulkes M.J., Sylvester-Bradley R., Scott R.K., 1998. Evidence for differences between winter wheat cultivars in aquisition of soil mineral nitrogen and utilization of applied fertilizer nitrogen. Journal of Agricultural Science 130, 29–44.

Gáborčík N., 2003. Relationship between contents of chlorophyll (a+b)(SPAD values) and nitrogen of some temperate grasses. Photosynthetica 41, 2855–287.

Giunta F., Motzo R., Deidda M., 2002. Spad readings and associated leaf traits in durum wheat, barley and triticale cultivars. Euphytica 125, 197–205.

Lafitte H.R., Edmeades G.O., Taba S., 1997. Adaptive strategies identified among tropical maize landraces for nitrogen-limited environments. Field Crop Research 49, 187 – 204.

Lammer D., Cai X., Arterburn M., Chatelain J., Murray T., Jones S., 2004. A single chromosome addition from *Thinopyrum elongatum* confers a polycarpic, perennial habit to annual wheat. Journal of Experimental Botany 55(403), 1715–1720.

Morris C.F., Paulsen G.M., 1985. Development of hard winter wheat after anthesis as affected by nitrogen nutrition. Crop Science 25, 1007–1010.

Murphy K.M., Campbell K.G., Lyon S.R., Jones S.S., 2007. Evidence of varietal adaptation to organic farming systems. Field Crops Research 102, 172–177.

Piaskowski J., 2006. Genetic characterization and chromosome transmission of perennial wheat lines. Master's thesis, Washington State University.

Sinclair T.R., 1998. Historical changes in harvest index and crop nitrogen accumulation. Crop Science 38(3), 638–643.

Singh V.P., Arora A., 2001. Intraspecific variation in nitrogen uptake and nitrogen

utilization efficiency in wheat (*Triticum aestivum* L.). Journal of Agronomy and Crop Science 186, 239–244.

Vidal I., Longeri L., Hétier J.M., 1999. Nitrogen uptake and chlorophyll meter measurements in spring wheat. Nutrient cycling in agroecosystems 55, 1–6.

Woodruff D., 1972. Cultivar variation in nitrogen uptake and distribution in wheat. Australian Journal of Experimental Agriculture and Animal Husbandry 12, 511–516.

Chapter 5

Analysis of Nitrogen Use Efficiency for Wheat Breeding Using Principal Component Analysis

Abstract

Nitrogen use efficiency is a complex quantitative trait where many of the component variables are correlated to one another. This creates difficulty in multivariate analysis due to the distortion of correlation coefficients among variables. Principal component analysis (PCA) is a statistical tool often used in the study of genetic diversity and in genetic conservation efforts. This method of analysis could be very useful to breeding programs in deciding which variables are of most importance for particular environments and breeding goals. This paper uses data from a study of winter wheat genotypes selected under different N regimes and grown under organic fertility in the field, and data from a greenhouse experiment of

historic and modern annual spring wheat genotypes, perennial wheat breeding lines and a series of *Triticum aestivum*/*Thinopyrum elongatum* chromosome addition lines to test the utility of PCA for breeding wheat varieties adapted to low-input and organic agriculture. Results showed that certain variables such as yield and total grain N are highly correlated, so that total grain N may not be a good index of grain yield and grain %N. The first three PCA components were able to separate historic and modern wheat genotypes in both the greenhouse and the field, and was useful to visualize the relationships among genotypes currently in the breeding program for organic wheat.

5.1 Introduction

Many of the variables related to nitrogen use efficiency (NUE) are correlated, particularly yield, biomass and nitrogen (N) concentration measurements. This presents problems in multivariate analysis due to multicollinearity, which distorts correlation coefficients and R^2 values. Principal component analysis (PCA) is a statistical tool that addresses multicollinearity by deriving a set of orthogonal independent principal components from the original variables. It is a data reduction tool that clarifies the relationships among characters and divides the total variance of the original characters into a smaller number of uncorrelated new variables (Wiley, 1981). It is most effective when the original variables are highly correlated, so that a

few principal components are sufficient to describe the variation present in the data. The resulting principal components (PC) are of interest because they reveal the underlying dimensionality of the data and also the degree of redundancy in the original variables (Manly, 1994). This allows more accurate interpretation of the relationships among the original variables and among genotypes. In a two or three dimensional scatterplot, differences in linear distances between points represent differences among factors and genotypes with minimal distortion (Mohammadi and Prasana, 2003)

PCA is also useful for comparing variables that have very different scales and variances. For example, grain yield has a much greater mean and range than does grain %N. Total grain N, the product of grain yield and grain %N, is therefore strongly influenced by yield and does not provide a very useful index of the two variables. PCA generally transforms the original variables to have a mean of zero and unit variance, so that each variable has equal weight in the analysis (Manly, 1994). As NUE is traditionally assessed using a combination of indices of variables that are correlated and on different scales, PCA may provide a useful alternative means of analysis.

Since each original variable is transformed to a mean of zero and variance of one, the total variance in the transformed data set will be equal to the number of original variables. The eigenvalues of the derived principal components are the amount of the total variance that they explain (Manly, 1994). A principal component

with an eigenvalue of 5, for example, would explain the equivalent variation of 5 original variables. The first PC has the largest variance, and the variance declines with each subsequent PC, since they are independent. The cumulative variances of all the PCs will add up to the total variance of the transformed data.

The PCs can be interpreted in terms of the original variables by looking at the factor loadings for each variable. These loadings are calculated in the process of deriving the PCs, which are linear combinations of the original variables such that the variance of the PC is as large as possible, the squared coefficients of each variable sum to one, and the PC is independent of all other PCs (Manly, 1994). Variables with large positive or negative loadings on a particular PC have a strong influence on that PC. These loadings help in interpreting what each PC represents and the relationship among variables. Experimental treatments or genotypes can be examined in a similar manner, by looking at the scores for each PC. Genotypes with similar scores for several PC will group together in a scatterplot, and have similar responses to the variables with large factor loadings on that PC. This allows similar genotypes to be grouped based on a large number of measured variables.

PCA has been used in characterizing germplasm for plant breeding and conservation. Ghafoor et al. (2001) used PCA to study 484 accessions of blackgram (*Vigna mungo* L. Hepper) germplasm. they found that the first PC was primarily related to yield components, while the second PC contrasted vegetative and reproductive development traits. From the results of their study, they decided that

PCA could be used to assess the genetic variation and relationships among genotypes based on agronomic traits, and to help choose accessions for hybridization and use in variety improvement.

Additive main effect and multiplicative interaction (AMMI) and genotype main effects and genotype by environment interaction (GGE) models are often used in the analysis of multi-locational and multi-year trial data. AMMI combines analysis of variance (ANOVA) to compare main effects with principle component analysis (PCA) to compare interactions between factors. AMMI models first break variation into genetic (G), environmental (E) and genotype by environment (GE) interactions. PCA is then used to break the GE into a series of components based on their importance (Gauch, 2006). GGE is also based on ANOVA and PCA and is useful for analyzing crossover and non-crossover interactions between genetic and environmental factors (Ma et al., 2004; Yan and Hunt, 2001). This provides advantages over simple ANOVA for large datasets, however, both models are generally used to analyze only one trait of interest, usually yield. PCA has the advantage of being able to analyze a set of genotypes for multiple variables at once and to show the relationships among these variables.

A study comparing maize landraces and modern varieties found that PCA separated the two groups based on the first two PC (Lafitte et al., 1997). The first PC was primarily associated with grain and biomass yield, as well as anthesis date in high N treatments and ear leaf area in low N treatments. PC 2 was negatively

associated with harvest index, which is one of the major traits that distinguishes the modern varieties and landraces. In two groups of landraces, the PCA identified similar traits as being important to performance in low N evironments, so traits of importance to survival in this type of environment may be stable (Lafitte et al., 1997). Within the groups of landraces and modern varieties, there were diverse combinations of yield, grain %N and senescence rates. The late maturing landraces could be a source of increased N uptake under N stress, but have poorer N partitioning relative to the early maturing landraces. The late maturing types could still be a source of genes for high grain %N in low N conditions, while modern varieties could contribute to higher yields (Lafitte et al., 1997).

The objectives of this paper are to assess the utility of PCA for the breeding program in terms of the ability to study the genetic diversity and relationships among breeding lines. The data from this study came from a diverse range of genetic material, tested under organic conditions and in the greenhouse. In addition, it was of interest to look at the relationships among measured variables to potentially eliminate measurements not well correlated to the traits of interest. Measurements that are highly correlated to each other could also be eliminated if they did not provide additional useful information.

5.2 Materials and Methods

Plant germplasm, experimental design, agronomic management and procedures are explained in Chapter 3 and Chapter 4.

Procedure PRINCOMP was used in SAS software version 8 (SAS Inc, Cary, NC). For the field study, PCA was conducted on all measured variables in the experiment, including preplant soil N, spring soil N, postharvest soil N, early plant vigor, biomass production in the 0.6 m sample, grain weight in the 0.6 m sample, grain yield (kg/ha), test weight, grain moisture, grain %N, total grain N (kg/ha), harvest index (HI), straw yield (kg/ha), biomass yield calculated as grain yield (kg/ha)/HI, and SPAD chlorophyll meter readings taken at the 8-9 leaf stage (SPAD1), pre-anthesis (SPAD2) and post-anthesis (SPAD3). PC scores were obtained for the measured variables, each genotype, the selection categories, locations and years. These were used in a biplot analysis of the relationship between the variables, genotypic and environmental factors.

In the greenhouse experiment, PCA was used to evaluate the relationship between measured variables at different growth stages and to compare annual and perennial genotypes. Samples were taken at the 6 leaf growth stage (6leaf), anthesis and maturity. Separate PCA analyses were done for the experiments with annual spring wheat varieties, addition lines and perennial lines, including all measured variables. A joint PCA analysis was also done combining data from the perennial

Table 5.1: Eigenvalues and proportion of total variation explained for each PC and factor loadings for original variables for field PCA

	Prin1	Prin2	Prin3	Prin4	Prin5
eigenvalue	4.881	3.384	2.295	1.417	1.069
proportion	0.2871	0.199	0.135	0.0834	0.0629
preplant soil N	0.056	0.424	-0.072	0.357	0.098
spring soil N	0.162	0.364	0.201	0.25	0.104
postharvest soil N	0.252	0.14	0.416	0.035	0.017
early vigor	0.177	0.147	-0.316	0.132	-0.234
row biomass	0.339	0.018	-0.17	0.041	-0.133
row grain weight	0.289	0.262	-0.241	-0.246	0.01
yield kg\ha	0.39	-0.08	-0.19	-0.13	0.16
test weight	-0.028	-0.015	0.198	0.093	0.828
grain moisture	0.052	-0.182	0.359	0.031	-0.161
grain %N	0	0.265	0.225	0.37	-0.309
total grain N	0.393	-0.007	-0.189	-0.007	0.099
HI	-0.012	0.404	-0.118	-0.434	0.161
straw yield (kg/ha)	0.25	-0.37	-0.04	0.33	0.06
biomass yield (kg/ha)	0.32	-0.32	-0.11	0.24	0.09
SPAD1	0.165	-0.213	0.275	-0.427	-0.123
SPAD2	0.296	0.119	0.322	-0.116	-0.017
SPAD3	0.289	0.065	0.311	-0.141	-0.102

and annual experiments. PCA scores were calculated for each measured variable and each genotype separately and also for the categories historic, modern and perennial. Variables included in the analysis were 6leaf SPAD (perennial only), 6leaf days after planting (DAP), 6leaf biomass, 6leaf %N, 6leaf total N (TN), anthesis SPAD, anthesis DAP, anthesis biomass, anthesis %N, anthesis TN, maturity DAP, straw weight, straw %N, straw TN, grain weight, grain %N and grain TN.

5.3 Results

5.3.1 Field study

Five principal components (PC) had eigenvalues above one, meaning that they explained more of the variation present in the data than one original variable. The first three PCs had eigenvalues above two, and these three components explain over 60% of the variation in the data (See Table 5.1).

Examining the factor loadings for the measured variables shows which are the most influential for each principal component. Those with factor loadings having an absolute value greater than 0.3 are described here. For PC 1, the row biomass, yield (kg/ha), total grain N (kg/ha) and biomass yield (kg/ha) had positive loadings. PC 2 had positive loadings for preplant soil N, spring soil N, and harvest index (HI), and negative loadings for straw yield (kg/ha) and biomass yield (kg/ha). For PC 3, postharvest soil N, grain moisture, SPAD2 and SPAD3 had positive loadings, and a negative loading for the early vigor rating. Preplant soil N, grain %N and straw yield (kg/ha) had positive loadings for PC 4, and HI and SPAD1 had negative loadings. PC 5 had a very high positive loading for test weight (0.8) and a negative loading for grain %N. PC 6 had positive loadings for the early vigor rating and grain moisture and a negative loading for row biomass and grain %N (see Table 5.1).

The correlation matrix among measured variables shows associations between

Table 5.2: Correlations among original variables in the PCA of the field experiment. Relatively high correlations between variables are shaded.

variable	preplant soil N	spring soil N	post-harvest soil N	early vigor	row biomass	row grain weight	yield kg\ha	test weight	grain moisture	grain %N	total grain N	HI	straw yield (kg/ha)	biomass yield (kg/ha)	SPAD1	SPAD2
spring soil N	0.64															
postharvest soil N	0.161	0.676														
early vigor	0.288	0.236	0.046													
row biomass	0.111	0.187	0.243	0.364												
row grain weight	0.349	0.331	0.215	0.406	0.756											
yield kg\ha	-0.011	0.083	0.27	0.351	0.582	0.573										
test weight	0.006	0.1	0.113	-0.234	-0.127	-0.132	-0.05									
grain moisture	-0.244	-0.045	0.256	-0.191	-0.031	-0.242	0.03	0.068								
grain %N	0.41	0.385	0.255	0.036	0.012	0.015	-0.25	-0.027	0.029							
total grain N	0.131	0.186	0.268	0.422	0.586	0.604	0.95	-0.067	0.033	-0.023						
HI	0.406	0.296	0.038	0.141	-0.134	0.51	0.07	-0.04	-0.321	0.061	0.117					
straw yield (kg/ha)	-0.264	-0.131	0.116	0.066	0.382	-0.072	0.54	0.007	0.169	-0.217	0.49	-0.664				
biomass yield (kg/ha)	-0.206	-0.081	0.15	0.177	0.48	0.117	0.73	-0.018	0.138	-0.254	0.687	-0.506	0.97			
SPAD1	-0.524	-0.076	0.366	-0.112	0.147	0.018	0.28	-0.007	0.283	-0.178	0.174	-0.159	0.25	0.27		
SPAD2	0.197	0.391	0.629	0.042	0.343	0.354	0.39	0.043	0.186	0.177	0.381	0.106	0.16	0.22	0.35	
SPAD3	0.053	0.279	0.565	0.046	0.333	0.314	0.39	0.009	0.206	0.193	0.39	0.048	0.19	0.25	0.358	0.728

these variables (see Table 5.2). Correlations with an r value greater than 0.5 ($R^2 > 0.25$) are shaded. Strong positive correlations were observed between row grain weight and row biomass (r= 0.756), grain yield and total grain N (r= 0.95), grain yield and biomass yield (r= 0.73), straw yield and biomass yield (r= 0.97) and SPAD2 and SPAD3 (r= 0.728). These are not surprising, as biomass is the sum of straw and grain weights, and total grain N is largely dependent on grain yield (Oury and Godin, 2007). Grain %N showed a negative correlation to grain yield of r= -0.25, which gives an R^2 of only 0.0625. Grain %N and grain TN are very weakly negatively correlated (r= -0.023). This is encouraging in terms of developing varieties for organic systems with both adequate protein and high yields. Another set of interesting correlations are those among grain yield, biomass yield, straw yield and harvest index. Biomass and straw yield are positively correlated with grain yield and negatively correlated with HI. HI and grain yield have an r value close to zero. It appears that in this experiment, conducted in certified organic fields, high grain yields were not dependent on having a high HI.

The strong correlations between SPAD2 and SPAD3 may mean that only one of these measurements is necessary. Spring soil N was positively correlated with both preplant soil N and postharvest soil N, so it may be possible to rely on a preplant and postharvest soil sample to capture most of the information from these three variables. Interestingly, both SPAD2 and SPAD3 are positively correlated with postharvest soil N, so higher SPAD meter readings may be an indication of surplus

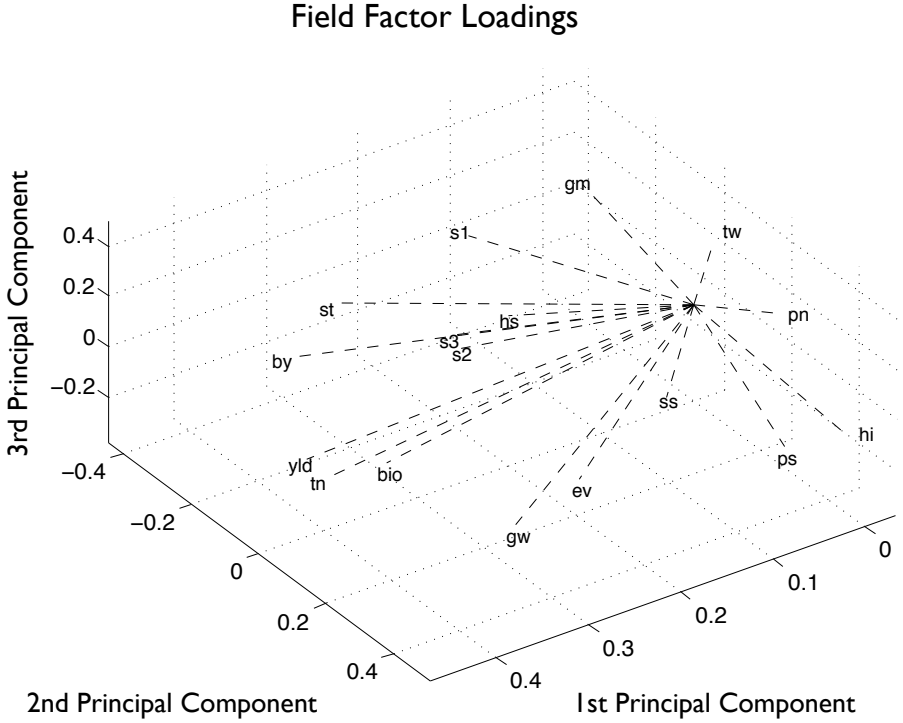


Figure 5.1: Plot of PC factor loadings for original variables in the field experiment
key: ps=preplant inorganic soil N; ss=spring inorganic soil N; hs=postharvest inorganic soil N; ev=early vigor; bio=row biomass; gw=row grain weight; yld=yield (kg/ha); tw=test weight; gm=grain moisture; pn=grain %N; tn=total grain N; hi=harvest index; st=straw yield (kg/ha); by=biomass yield (kg/ha); s1=SPAD1; s2=SPAD2; s3=SPAD3

soil N (residual inorganic N left in the soil after harvest). None of the SPAD readings were strongly correlated with grain yield, grain TN or grain %N.

From the plot of field factor loadings (Figure 5.1, it is clear that yield, total grain N and row biomass are closely related. SPAD3 and SPAD2 have similar scores, and are close to postharvest soil N. Leaf N concentration is closely correlated to leaf chlorophyll content so lines with high SPAD values would also have higher leaf N concentrations (Lee et al., 1999; Vidal et al., 1999; Gáborčík, 2003; Giunta et al.,

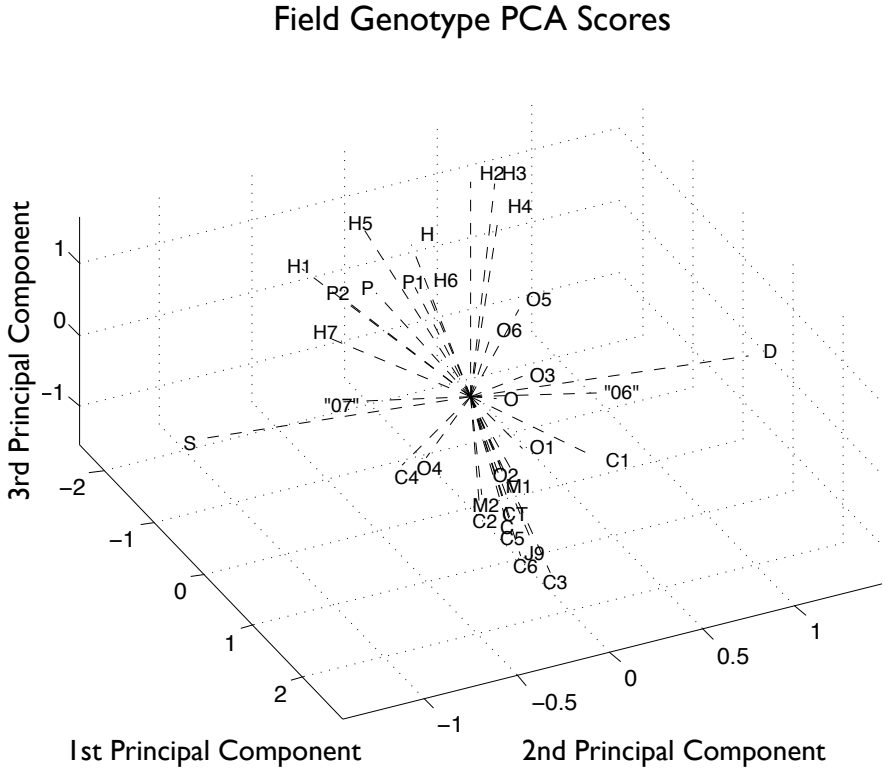


Figure 5.2: Plot of genotype PC scores in the field experiment
key: CT=control; C=conventional; H=historic; O=organic; P=perennial; S=Spillman; D=St. John; "06"=2006; "07"=2007; J9=J99C0009; M1=Madsen1; M2=Madsen2; C1=4J020185; C2=4J020187; C3=4J020210-6; C4=4J020259; C5=4J020274; C6=4J020275; H1=Bunyip; H2=Hyper; H3=Idaed; H4=Onas; H5=Sonora; H6=WhiteMarquis1; H7=WhiteMarquis2; O1=5K020007; O2=5K020023; O3=5K020082; O4=5K020095; O5=5K020106; O6=5K020138; P1=Perennial Bulk1; P2=Perennial Bulk2

2002; Olfs et al., 2005; Wolfe et al., 1988). Grain weight and early vigor are also close to each other. Other measured variables are more dispersed on the plot of the first 3 PCs.

Plotting genotype, category, location and year scores (Figure 5.2) shows that Spillman and the DeLongs farm are opposite each other for each PC. The years 2007 and 2006 are also opposing, with 2007 being closer to the scores for Spillman and

2006 being closer to the scores for DeLong. Historic and perennial genotypes group to one side, with the perennial and historic category means, and conventional and control group to the other, with the means for these two categories. Three organic genotypes group with the conventional and control genotypes and three group closer to the historic and perennial genotypes, although their scores are closer to the origin, on the same side as the DeLongs and 2007. The organic mean is near the origin.

5.3.2 Greenhouse eigenvalues and factor loadings

For all four PCA, there were 5 principal components (PC) above one, meaning that they explained more of the variation in the data set than one original variable. In the annual experiment, the first 5 PC explain 77.43% of the total variation. For the addition lines, the first 5 PC explain 79.29% of the total variation. For the perennial lines, the first 5 PC explain 78.63% of the total variation. In the joint PCA of annual and perennial lines, the the first 5 PC explain 79.44% of the total variation. Table 5.3, Table 5.4, Table 5.5 and Table 5.6 show the eigenvalues of each PC, the proportion of the total variation that PC explains and the variable factor loadings, with the most influential identified in yellow. While the variables important to each principal component differ from one analysis to the next, the number of principal components with eigenvalues above one is the same for all analyses, and the proportion of variation explained by these PC is similar.

Table 5.3: Eigenvalues, proportion of variation explained and factor loadings for PCA of annual variety experiment

	Prin1	Prin2	Prin3	Prin4	Prin5
Eigenvalue	5.375	2.068	1.713	1.394	1.065
Proportion	0.3583	0.1379	0.1142	0.0929	0.071
6leaf DAP	-0.076	0.321	0.44	-0.056	0.08
6leaf biomass	-0.312	0.094	0.466	-0.09	-0.073
6leaf %N	0.297	-0.017	-0.177	0.13	0.214
6leaf TN	-0.277	0.102	0.473	-0.045	0.006
anthesis DAP	0.095	0.37	-0.098	0.446	-0.156
anthesis biomass	-0.206	0.201	-0.081	0.508	-0.414
anthesis %N	0.354	-0.134	0.25	-0.194	0.111
anthesis TN	0.309	0.029	0.272	0.269	-0.286
maturity DAP	-0.135	0.287	-0.141	0.074	0.428
straw weight	-0.186	-0.127	0.122	0.445	0.528
straw %N	0.328	0.33	0.039	-0.127	0.011
straw TN	0.27	0.244	0.144	0.202	0.417
grain weight	0.109	-0.512	0.223	0.313	-0.004
grain %N	0.318	0.323	0.067	-0.137	-0.101
grain TN	0.336	-0.22	0.264	0.162	-0.081

Table 5.4: Eigenvalues, proportion of variation explained and factor loadings for PCA of addition line experiment

	Prin1	Prin2	Prin3	Prin4	Prin5
Eigenvalue	4.2651	3.7075	2.0699	1.516	1.128
Proportion	0.2666	0.2317	0.1294	0.0948	0.0705
6leaf DAP	0.1802	-0.061	-0.3129	0.5342	-0.008
6leaf biomass	-0.1035	-0.4052	-0.1936	0.2376	0.3352
6leaf %N	0.0366	-0.0027	0.3211	-0.3324	0.4199
6leaf TN	-0.0968	-0.4153	-0.1658	0.2049	0.3614
anthesis SPAD	0.2985	0.1367	-0.1862	-0.0915	-0.2048
anthesis DAP	-0.3392	0.0373	-0.1449	0.2978	-0.2303
anthesis biomass	-0.3638	0.1213	0.237	0.148	0.2355
anthesis %N	0.4283	0.1414	-0.1068	0.16	-0.037
anthesis TN	0.1188	0.325	0.1694	0.3576	0.2425
maturity DAP	-0.241	0.289	-0.092	0.144	-0.3
straw weight	-0.3283	0.2256	0.2025	0.185	0.0059
straw %N	0.2097	0.3034	-0.1532	-0.0109	0.3272
straw TN	-0.1082	0.4351	0.0109	0.1394	0.2731
grain weight	0.2537	-0.1338	0.4854	0.2711	-0.1153
grain %N	0.113	0.2484	-0.3732	-0.1215	0.2916
grain TN	0.3368	-0.0628	0.3662	0.2653	-0.005

Table 5.5: Eigenvalues, proportion of variation explained and factor loadings for PCA of perennial line experiment

	Prin1	Prin2	Prin3	Prin4	Prin5
eigenvalues	5.7228	3.0012	1.9985	1.6261	1.0178
Proportion	0.3366	0.1765	0.1176	0.0957	0.0599
6leaf SPAD	0.1294	0.1684	0.3003	0.2918	-0.0274
6leaf DAP	-0.2053	0.0867	0.3533	0.0766	0.4536
6leaf biomass	-0.3657	0.0397	0.1188	0.1896	0.1559
6leaf %N	-0.1655	-0.026	-0.016	-0.4118	0.579
6leaf TN	-0.3701	0.0403	0.1176	0.1674	0.179
anthesis SPAD	0.2515	-0.0515	-0.1224	0.2727	0.4416
anthesis DAP	0.2933	0.0791	-0.0592	-0.257	-0.0522
anthesis biomass	0.0188	0.3342	0.1451	-0.532	-0.044
anthesis %N	0.2554	-0.3237	-0.2264	0.193	0.2298
anthesis TN	0.3185	-0.0592	-0.2312	-0.2152	0.3046
maturity DAP	0.244	0.126	0.295	-0.222	0.088
straw weight	0.1368	0.4132	0.2353	0.0812	0.0549
straw %N	0.1734	-0.3927	0.3604	0.0012	-0.1124
straw TN	0.2388	-0.1427	0.5036	0.0439	-0.0447
grain weight	0.2422	0.3847	-0.111	0.2325	0.0774
grain %N	0.1384	-0.3803	0.2723	-0.0807	0.1147
grain TN	0.2888	0.2785	-0.0106	0.2234	0.1095

Table 5.6: Eigenvalues, proportion of variation explained and factor loadings for joint PCA of annuals and perennials

	Prin1	Prin2	Prin3	Prin4	Prin5
Eigenvalue	4.874	2.506	1.985	1.378	1.173
Proportion	0.3249	0.1671	0.1324	0.0918	0.0782
6leaf DAP	-0.296	0.291	-0.03	0.348	0.102
6leaf biomass	-0.381	0.014	-0.019	0.193	0.389
6leaf %N	0.126	0.262	-0.123	0.002	-0.564
6leaf TN	-0.378	0.075	-0.043	0.203	0.27
anthesis DAP	-0.08	0.209	0.066	0.596	-0.313
anthesis biomass	-0.075	-0.356	0.268	0.307	-0.344
anthesis %N	0.269	0.414	-0.098	-0.095	0.25
anthesis TN	0.297	0.203	0.157	0.142	-0.044
maturity DAP	0.146	-0.244	0.26	0.32	0.084
straw weight	0.105	-0.335	0.434	-0.095	0.136
straw %N	0.336	0.014	-0.262	0.322	0.202
straw TN	0.356	-0.195	0.04	0.231	0.272
grain weight	-0.037	0.352	0.545	-0.103	0.067
grain %N	0.355	-0.039	-0.238	0.204	0.105
grain TN	0.191	0.351	0.437	0.019	0.107

5.3.3 Relationship among genotypes and variables in the greenhouse

Table 5.7 and Table 5.8 show the correlation coefficients between variables for each experiment and the joint PCA of perennials and annuals. Correlations greater than 0.5 ($R^2 > 0.25$) are shaded.

In the addition line experiment, 6leaf TN and biomass show a strong positive correlation, but traits measured at 6leaf do not show very high correlations with anthesis or maturity traits. Anthesis SPAD measurements are positively correlated with anthesis %N, but not with grain or straw traits. Biomass at anthesis is negatively correlated with anthesis %N and positively correlated with straw weight, but not with grain weight. Anthesis %N is positively correlated with straw %N and grain TN, and anthesis TN is positively correlated with straw TN. At maturity, DAP is positively correlated both with straw weight and with straw TN, and straw %N, TN and weight are all positively correlated. Grain weight shows a strong positive correlation with grain TN. The correlation between grain weight and grain %N is near zero.

For the perennial lines, there is a positive correlation between 6leaf DAP, biomass and TN. 6leaf biomass is negatively correlated to anthesis DAP, %N, and TN. There is a negative correlation between 6leaf TN and grain TN, but no strong correlation between other traits at the 6leaf stage or at anthesis and those at

maturity. Among traits measured at maturity, DAP is positively correlated to straw weight, and straw weight is positively correlated to both grain weight and grain TN. Straw %N and TN are both positively correlated to grain %N, and there is a strong positive correlation between grain weight and grain TN.

For the annual experiment, 6leaf biomass is strongly correlated to 6leaf TN and negatively correlated to 6leaf %N, however, traits measured at the 6leaf stage showed low correlation to traits at anthesis or maturity. Biomass at anthesis also showed low correlations in general to maturity traits, although it is interesting to note that these correlations are negative for all maturity traits except DAP and straw weight. In contrast, total N and %N at anthesis were correlated to grain %N, grain total N, straw %N and to a lesser extent, straw TN and grain weight. Grain TN is also positively correlated to grain weight and grain %N.

In the comparison of annual and perennial genotypes, 6leaf biomass is negatively correlated to grain %N, and 6leaf total N is negatively correlated with straw TN and grain %N. 6leaf biomass shows a strong positive correlation to 6leaf TN as in the annual experiment alone. DAP at the 6leaf stage is positively correlated to both 6leaf biomass and TN. At the anthesis stage, %N was negatively correlated with biomass and positively correlated to TN. Anthesis TN was positively correlated with grain TN, but other traits at anthesis did not show a strong correlation to maturity traits. At maturity, straw %N was strongly correlated to straw TN, and both straw %N and TN were positively correlated to grain %N. As in

Table 5.7: Correlations among original variables in the PCA of the greenhouse experiments with chromomsome addition lines and perennial lines.
Correlations for the chromosome addition line experiment are shown below the diagonal, those for the perennial line experiment are shown above the diagonal

Variable	6leaf DAP	6leaf biomass	6leaf %N	6leaf TN	anthesis SPAD	anthesis DAP	anthesis biomass	anthesis %N	anthesis TN	maturity DAP	straw weight	straw %N	straw TN	grain weight	grain %N	grain TN
6leaf SPAD *	0.0362	-0.1196	-0.2425	-0.1232	0.2316	0.1311	0.0483	-0.0223	0.009	0.315	0.326	0.108	0.3608	0.3158	-0.051	0.3399
6leaf DAP	1	0.5677	0.2763	0.5692	-0.2515	-0.324	0.0614	-0.4085	-0.4257	-0.076	0.0713	-0.0892	-0.0299	-0.1515	-0.0123	-0.1498
6leaf biomass	0.255	1	0.2271	0.9979	-0.373	-0.5943	-0.0897	-0.5232	-0.6811	-0.494	-0.1263	-0.3403	-0.3791	-0.4241	-0.2442	-0.4984
6leaf %N	-0.2368	-0.1416	1	0.2761	-0.2168	-0.2462	0.2041	-0.1975	-0.0457	-0.057	-0.1905	-0.1829	-0.2438	-0.3353	-0.0619	-0.3491
6leaf TN	0.2338	0.9946	-0.0643	1	-0.3783	-0.6049	-0.0744	-0.5318	-0.6811	-0.491	-0.1318	-0.348	-0.3869	-0.4353	-0.2479	-0.5101
anthesis SPAD	0.181	-0.2845	-0.133	-0.2918	1	0.292	-0.2016	0.5712	0.538	0.209	0.123	0.1705	0.2508	0.3785	0.1859	0.4154
anthesis DAP	0.1212	0.1211	-0.2306	0.0888	-0.3925	1	0.3127	0.2744	0.6634	0.394	0.2201	0.1585	0.2709	0.3809	0.1646	0.4342
anthesis biomass	-0.3771	0.0154	0.0458	0.0043	-0.4601	0.4819	1	-0.5857	0.1317	0.315	0.3715	-0.2371	-0.0111	0.1686	-0.1706	0.1167
anthesis %N	0.4774	-0.3079	-0.0944	-0.3135	0.5984	-0.5038	-0.6682	1	0.663	0.111	-0.2082	0.426	0.2793	0.1025	0.3915	0.2144
anthesis TN	0.1403	-0.3961	-0.0322	-0.408	0.1936	-0.1346	0.2685	0.4495	1	0.344	0.0667	0.1762	0.2081	0.335	0.2538	0.4013
maturity DAP	-0.0343	-0.3071	-0.1268	-0.336	-0.101	0.5663	0.3194	-0.2156	0.0874	1	0.4555	0.2351	0.5008	0.2952	0.1555	0.3415
straw weight	-0.2789	-0.1697	-0.0214	-0.1816	-0.3033	0.4124	0.6969	-0.445	0.2466	0.546	1	-0.2358	0.331	0.6079	-0.225	0.5399
straw %N	0.1553	-0.3594	0.0628	-0.3691	0.3026	-0.2408	-0.2223	0.5251	0.3568	0.091	-0.2243	1	0.8025	-0.2392	0.7137	-0.0145
straw TN	-0.1163	-0.4142	-0.0037	-0.4336	0.0215	0.1528	0.3949	0.0495	0.5328	0.528	0.6101	0.5797	1	0.0674	0.5381	0.26
grain weight	0.1043	-0.0323	0.1719	-0.0113	0.0556	-0.3565	-0.2034	0.3372	0.1877	-0.349	-0.2026	-0.0619	-0.2672	1	-0.2905	0.9272
grain %N	0.1764	-0.237	-0.0031	-0.2523	0.3402	-0.0784	-0.1384	0.3577	0.2523	0.085	-0.1097	0.4334	0.2868	-0.4552	1	0.0154
grain TN	0.2481	-0.1071	0.2059	-0.0893	0.2132	-0.4074	-0.3032	0.5308	0.2787	-0.37	-0.3035	0.1168	-0.2108	0.9071	-0.0989	1

*** 6leaf SPAD readings for perennial experiment only**

the annual experiment, grain weight and grain TN are strongly correlated.

Figure 5.3 shows the relationship among variables for the annual genotype experiment. Scores for the first three PC are plotted. It is clear that 6leaf weight and TN have similar factor loadings, opposite 6leaf %N. Grain %N and straw %N also fall very close to one another, and grain TN is close to anthesis TN and anthesis %N. Straw TN is between these two groups, while straw weight, grain weight and anthesis biomass are on the opposite side. Interestingly, DAP for the three stages are not clustered together at all, suggesting that these three measurements are not well correlated.

Figure 5.4 shows the relationship among genotypes. Mean scores for the historic and modern categories are also plotted for the first three PC. Modern and historic categories fall opposite each other, with most of their respective genotypes grouping around the category mean. Exceptions to this are Spinkota, an historic genotype that is close to the modern mean. Canus and White Federation are two other historic genotypes which fall among the modern ones. No modern genotypes cluster near the historic mean.

In the addition lines, 6leaf TN and biomass have similar factor loadings for PC 1, 2 and 3. Anthesis %N and SPAD group fairly close to straw %N and grain %N. Anthesis biomass is close to straw weight. Grain weight and grain TN are close to each other but separated from other measured traits (see Figure 5.5).

Table 5.8: Correlations among variables in the PCA of the greenhouse experiments with annual genotypes and in the joint PCA of annual genotypes and perennial lines
Correlations for the annual experiment are shown below the diagonal, those for the joint analysis of annual and perennial lines are shown above

Variable	6leaf DAP	6leaf biomass	6leaf %N	6leaf TN	anthesis DAP	anthesis biomass	anthesis %N	anthesis TN	maturity DAP	straw weight	straw %N	straw TN	grain weight	grain %N	grain TN
6leaf DAP	1	0.653	0.008	0.691	0.423	-0.071	-0.096	-0.249	-0.188	-0.405	-0.291	-0.492	0.218	-0.426	-0.041
6leaf biomass	0.394	1	-0.421	0.954	0.127	0.096	-0.378	-0.449	-0.21	-0.164	-0.45	-0.489	0.048	-0.516	-0.301
6leaf %N	-0.179	-0.693	1	-0.186	0.156	-0.191	0.29	0.225	-0.084	-0.194	0.154	0.003	0.028	0.18	0.161
6leaf TN	0.394	0.927	-0.418	1	0.174	0.058	-0.351	-0.446	-0.246	-0.2	-0.449	-0.515	0.067	-0.509	-0.277
anthesis DAP	0.095	-0.174	0.18	-0.148	1	0.124	-0.055	0.038	0.024	-0.22	0.005	-0.131	0.153	-0.087	0.125
anthesis biomass	0.071	0.271	-0.29	0.242	0.241	1	-0.654	0.069	0.221	0.308	-0.187	0.016	-0.08	-0.124	-0.154
anthesis %N	-0.024	-0.407	0.44	-0.367	-0.036	-0.7	1	0.64	-0.08	-0.199	0.462	0.278	0.192	0.431	0.474
anthesis TN	0.054	-0.325	0.399	-0.277	0.254	-0.015	0.656	1	0.136	0.054	0.395	0.372	0.197	0.461	0.506
maturity DAP	0.226	0.136	-0.087	0.14	0.202	0.121	-0.327	-0.317	1	0.392	0.2	0.45	-0.004	0.169	0.097
straw weight	0.033	0.282	-0.184	0.296	-0.078	0.244	-0.31	-0.24	0.086	1	-0.126	0.469	0.122	0.021	0.158
straw %N	0.016	-0.398	0.412	-0.36	0.26	-0.272	0.535	0.499	-0.089	-0.506	1	0.772	-0.292	0.76	0.156
straw TN	0.038	-0.312	0.396	-0.258	0.237	-0.184	0.459	0.468	-0.059	0.124	0.742	1	-0.176	0.657	0.229
grain weight	-0.185	-0.138	0.16	-0.114	-0.106	-0.177	0.327	0.31	-0.208	0.113	-0.145	-0.014	1	-0.397	0.765
grain %N	0.062	-0.377	0.429	-0.312	0.3	-0.268	0.534	0.51	-0.166	-0.434	0.746	0.542	-0.242	1	0.202
grain TN	-0.087	-0.402	0.461	-0.339	0.106	-0.366	0.712	0.673	-0.334	-0.223	0.411	0.394	0.669	0.502	1

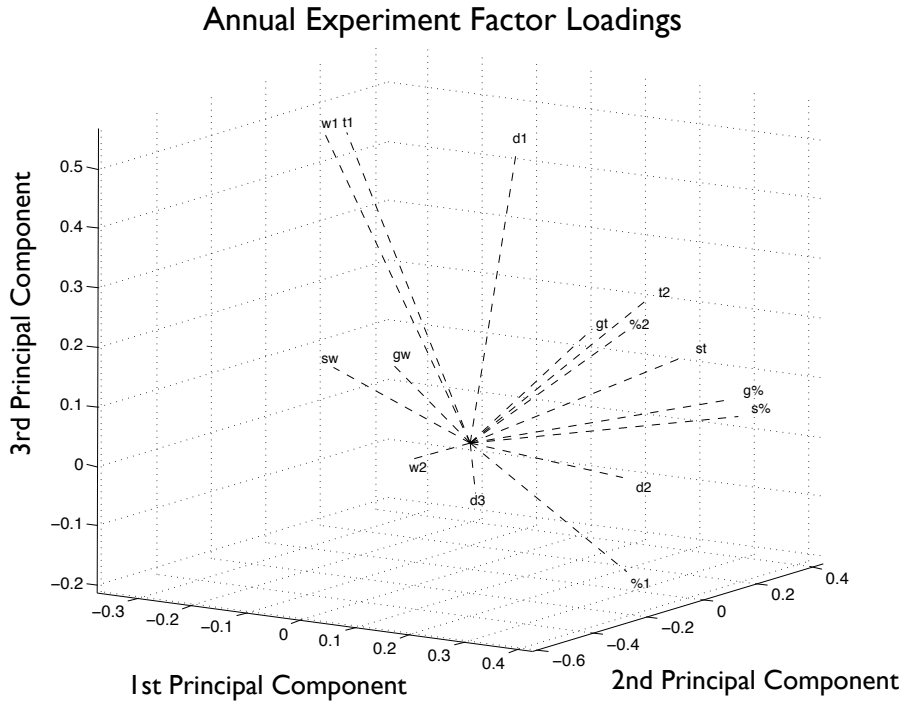


Figure 5.3: Plot of PC factor loadings for original variables in the greenhouse experiment with annual genotypes

Key: d1=6leaf DAP; w1=6leaf weight; %1=6leaf %N; t1=6leaf TN; d2=anthesis DAP; w2=anthesis biomass; %2=anthesis %N; t2=anthesis TN; d3=maturity DAP; sw=straw weight; s%=straw %N; st=straw TN; gw=grain weight; g%=grain %N; gt=grain TN

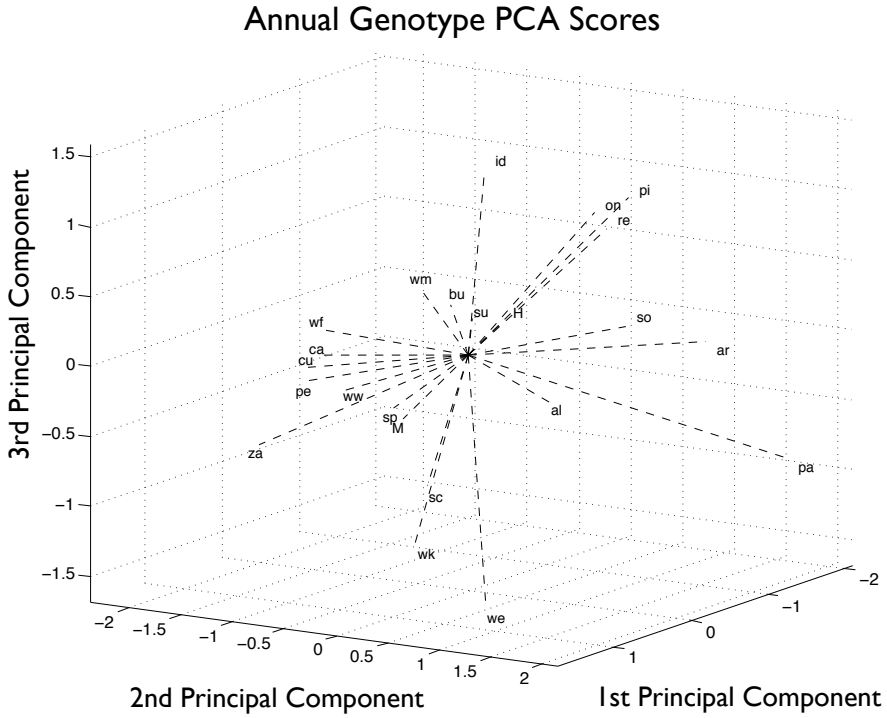


Figure 5.4: Plot of PC scores for annual genotypes.
Key: H=historic; M=modern; al=Alpowa (M); ar=Arco (H); bu=Bunyip (H); ca=Canus (H); cu=Currawa (M); id=Idaed (H); on=Onas (H); pa=Pacific Bluestem (H); pe=Penawawa (M); pi=Pilcraw (H); re=Red Fife (H); sc=Scarlet (M); so=Sonora (H); sp=Spinkcota (H); su=Surprise (H); wk=Wakanz (M); ww=Wawawai (M); we=Westbred Express (M); wf=White Federation (H); wm=White Marquis (H); za=Zak (M)

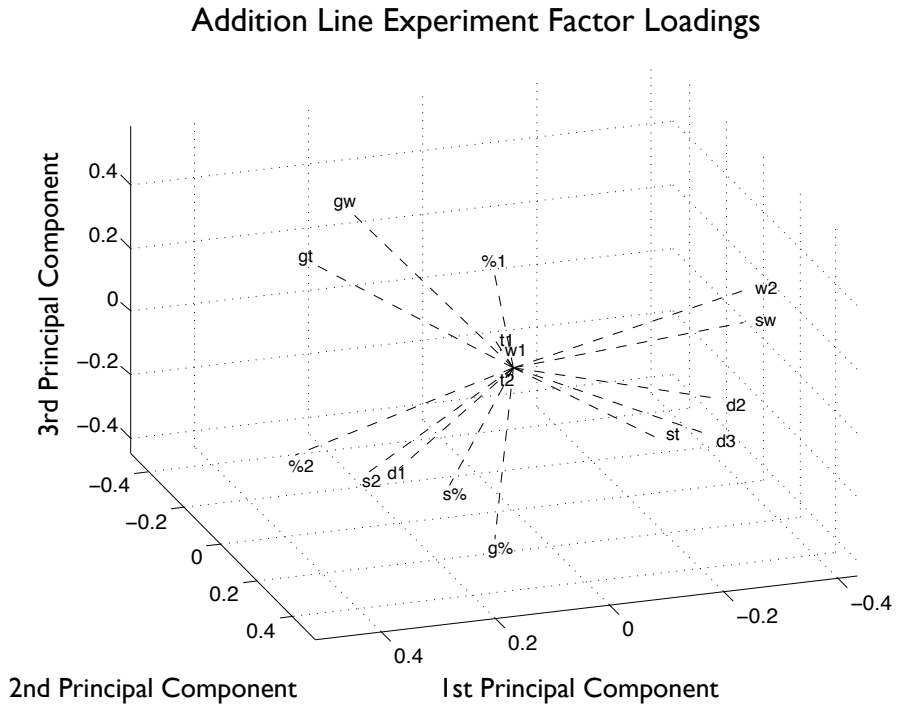


Figure 5.5: Plot of PC factor loadings for original variables in the greenhouse experiment with chromosome addition lines and parental genotypes
Key: d1=6leaf DAP; w1=6leaf weight; %1=6leaf %N; t1=6leaf TN; s2=anthesis SPAD; d2=anthesis DAP; w2=anthesis biomass; %2=anthesis %N; t2=anthesis TN; d3=maturity DAP; sw=straw weight; s%=straw %N; st=straw TN; gw=grain weight; g%=grain %N; gt=grain TN

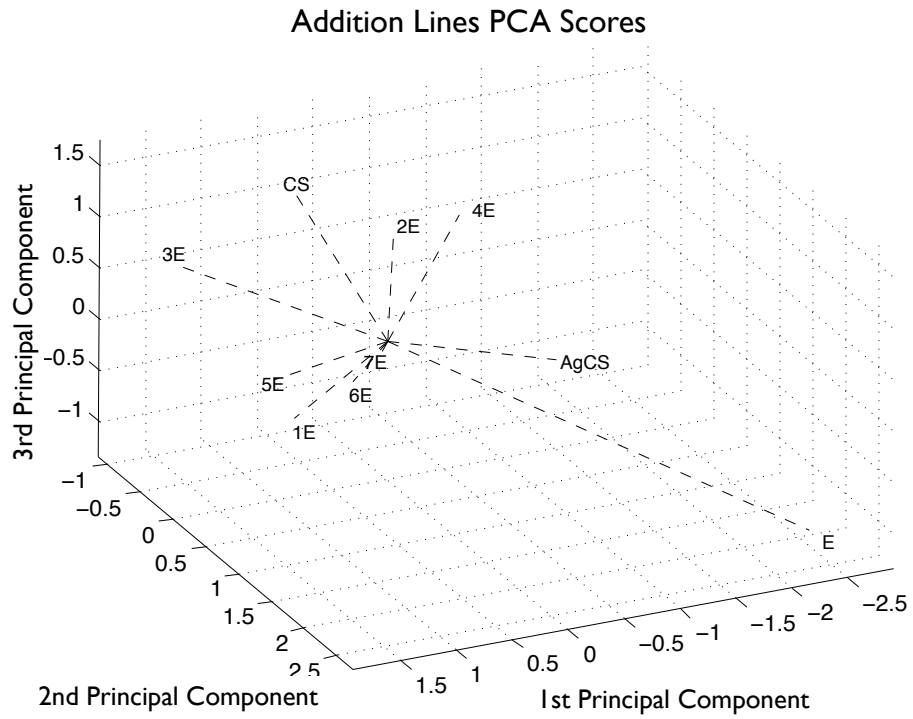


Figure 5.6: Plot of PC scores for chromosome addition lines and parental genotypes. Key: AgCS=AgCs; CS=CS; 1E=CS+1E; 2E=CS+2E; 3E=CS+3E; 4E=CS+4E; 5E=CS+5E; 6E=CS+6E; 7E=CS+7E; E=Elongatum

For the addition genotypes, *Th. elongatum* has the most negative scores on PC 1 and PC 3 and the most positive on PC 2. AgCS has scores similar to *Th. elongatum* for PC 1 and PC 3, but falls opposite on PC 2, with a negative score. CS has a moderate positive scores for PC 1, a moderate negative score for PC 2 and a large positive score for PC 3. CS+4E is the only genotype with a larger positive score than CS for PC 3, and has a moderate positive score for PC 2 and a moderate negative score for PC 1 (see Figure 5.6). *Th. elongatum* is a perennial wild wheat grass and CS+4E shows a perennial growth habit, while the rest of the chromosome addition lines are annuals (Lammer et al., 2004).

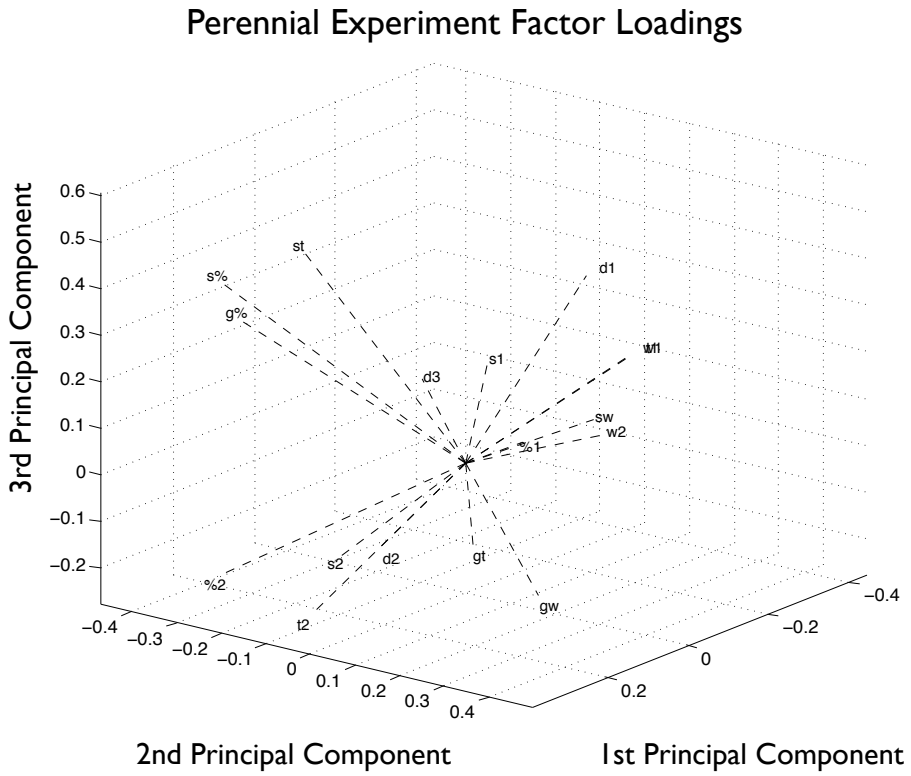


Figure 5.7: Plot of PC factor loadings for original variables in the greenhouse experiment with perennial lines
Key: s1=6leaf SPAD; d1=6leaf DAP; w1=6leaf weight; %1=6leaf %N; t1=6leaf TN; s2=anthesis SPAD; d2=anthesis DAP; w2=anthesis biomass; %2=anthesis %N; t2=anthesis TN; d3=maturity DAP; sw=straw weight; s%=straw %N; st=straw TN; gw=grain weight; g%=grain %N; gt=grain TN

Figure 5.7 shows that for the perennial lines, 6leaf biomass and TN fall nearly on top of each other, while anthesis biomass and anthesis TN are separated, with TN being more closely related to anthesis %N, SPAD reading and DAP, and anthesis biomass more closely related to straw weight at maturity. Straw TN, straw %N and grain %N cluster together, separated from grain weight and grain TN.

The perennial genotypes show a very interesting pattern in the first 3 PCs, as

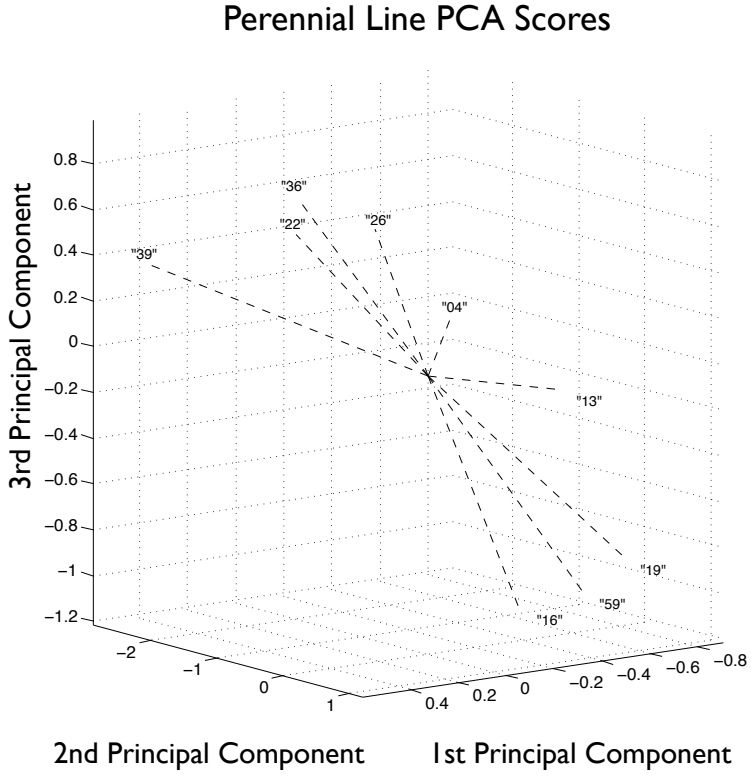


Figure 5.8: Plot of PC scores for perennial lines.
Key: "04"=03JP004; "16"=03JP016; "19"=03JP019; "22"=03JP022; "26"=03JP026; "36"=03JP036; "39"=03JP039; "13"=PI550713; "59"=SS259

they fall along a plane in three dimensions. 03JP016 and 03JP019 group with SS259, while PI550713 and 03JP004 are separated from these lines and the other four breeding lines (see Figure 5.8).

The joint analysis of annual and perennial genotypes showed both similarities and differences in the factor loadings compared to the individual perennial and annual analyses. Biomass and TN at the 6leaf stage have similar loadings, this time close to both 6leaf and anthesis DAP. Anthesis biomass and straw weight group together, like for the perennial analysis but unlike the annuals. Grain %N and straw

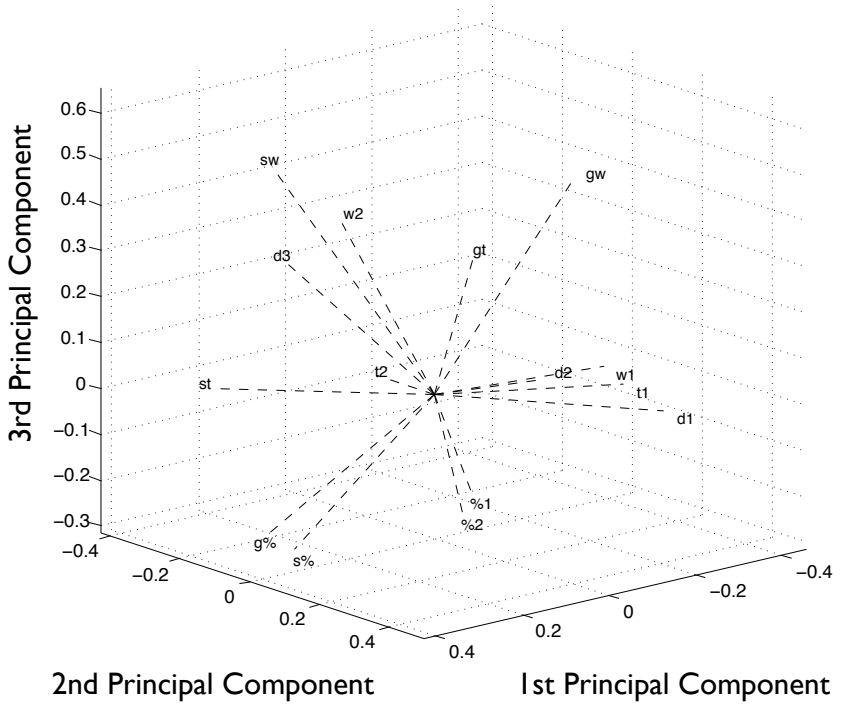


Figure 5.9: Plot of PC factor loadings for original variables in the joint analysis of the greenhouse experiments with annual genotypes and perennial lines
Key: d1=6leaf DAP; w1=6leaf weight; %1=6leaf %N; t1=6leaf TN; d2=anthesis DAP; w2=anthesis biomass; %2=anthesis %N; t2=anthesis TN; d3=maturity DAP; sw=straw weight; s%=straw %N; st=straw TN; gw=grain weight; g%=grain %N; gt=grain TN

%N have similar loadings, as in both the annual and perennial analyses, again separated from grain weight and grain TN. In this analysis 6leaf %N and anthesis %N have similar scores, while in the perennial and annual analyses they were separated (see Figure 5.9).

Figure 5.10 shows the mean scores for the categories historic, modern and perennial, as well as individual genotype scores for the first three PC. The historic and modern means are more similar than the perennial mean, and all the perennial

genotypes cluster on one side of the plot with their mean. The annual genotypes fall on the other side of the plot, and group roughly by category. Exceptions to this are Wawawai, which falls among the historic genotypes, and White Federation, which groups with the modern genotypes. Red Fife and Idaed are on the border of the modern and historic genotypes. Pacific Bluestem is the historic genotype and Westbred Express is the modern genotype with scores closest to the perennial lines.

5.4 Discussion

5.4.1 Field study

PC 1 appears to be a measure of biomass and grain production. This agrees with the results of both Ghafoor et al. (2001) in blackgram and Lafitte et al. (1997) in maize. It may be that, in field studies, these traits are typically the most variable of agronomic characters among a diverse group of genotypes. Total grain N was strongly correlated to grain yield, which confirms the results of the regression analysis in Chapter 3 and those reported in the literature (Loffler et al., 1985; Dhugga and Waines, 1989; Bertin and Gallais, 2000). As biomass measures were correlated to row grain weight and straw yield, it is likely that genotypes with high scores for PC 1 have high yield, total grain N, straw yield, total biomass, row biomass and row grain weight. Grain %N was not associated with PC1. Costa and Kronstad (1994) stated that by increasing biomass yield, total plant N uptake would

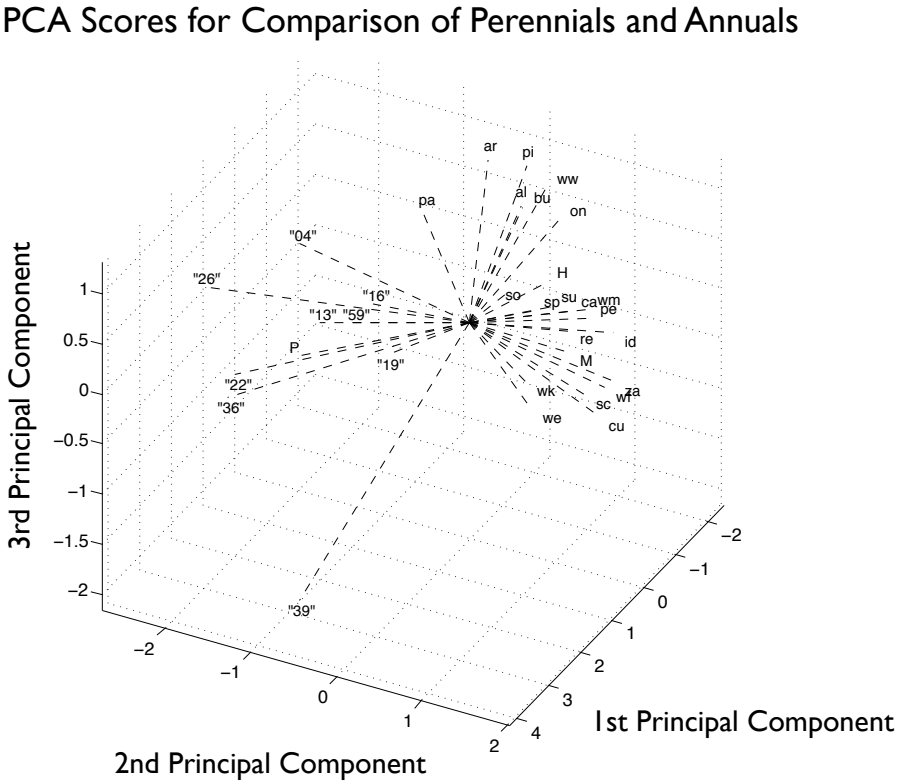


Figure 5.10: Plot of PC scores for joint analysis of annual genotypes and perennial lines.
Key: H=historic; M=modern; P=perennial; al=Alpowa (M); ar=Arco (H); bu=Bunyip (H); ca=Canus (H); cu=Currawa (M); id=Idaed (H); on=Onas (H); pa=Pacific Bluestem (H); pe=Penawawa (M); pi=Pilcraw (H); re=Red Fife (H); sc=Scarlet (M); so=Sonora (H); sp=Spinkcota (H); su=Surprise (H); wk=Wakanz (M); ww=Wawawai (M); we=Westbred Express (M); wf=White Federation (H); wm=White Marquis (H); za=Zak (M); "04"=03JP004; "16"=03JP016; "19"=03JP019; "22"=03JP022; "26"=03JP026; "36"=03JP036; "39"=03JP039; "13"=PI550713; "59"=SS259

increase and more N would be available for redistribution to grain. However, in the crosses they studied, there was generally no association between grain protein concentration and biological yield or non-grain biomass (Costa and Kronstad, 1994). In this study, there were very weak negative correlations between biomass and straw weight and grain %N. It appears that unless cultivars are specifically selected for higher grain %N, increases in biomass will not increase the N concentration in grain, although grain yield may increase.

PC 2 includes both the N available to the plant during the growing season and the HI. Both straw yield and biomass yield have negative loadings for this component, reflecting their negative correlation with HI. Desai and Bhatia (1978) found that HI was positively correlated with grain yield and grain %N, however, in this study neither of these traits was strongly correlated with HI. While total biomass does not always show a negative correlation to HI, in low N situations total biomass production may be increased by decreasing the HI, since straw has a lower N concentration than grain, so plants are able to produce greater amounts of biomass with limited N supplies if most of this biomass is straw rather than grain (Sinclair, 1998).

PC 3 has positive loadings for postharvest soil N, grain moisture, SPAD2 and SPAD3, and a negative loading for early vigor. Early vigor has a moderate positive correlation with biomass, yield and total grain N, so it is possible that lines with poor early growth had lower vegetative biomass and higher tissue N concentrations

throughout the season. Historic varieties often had lower early season vigor, probably because they are genetically spring wheat types which have been historically grown as winter wheat. Higher values of inorganic soil N post harvest could be due to lower uptake in blocks where plant growth was poor, or may have been an indication of surplus soil N, promoting luxury consumption, higher tissue N concentrations (and thus higher SPAD readings) and later senescence because of adequate nutrient status.

While care must be used in interpreting PC scores, based on the factor loadings, genotypes with positive loadings for PC 1, negative or neutral loadings for PC 2 and negative loadings for PC 3 may be the most efficient in terms of N use in an organic system. Positive PC 1 scores would indicate an ability to produce biomass and grain yields, negative PC 2 scores would also be indicators of good biomass production, especially with low preplant and spring soil inorganic N. As HI is not correlated with yield, this trait may not be as important in an organic system as in conventional systems. Studies of modern and historic cultivars in conventional systems have found that HI is highly correlated with increases in yield (Sinclair, 1998). In contrast, good vegetative growth and biomass production in organic systems may increase weed competitiveness and vegetative tissue may serve as an N source for developing grain when soil N supplies are exhausted.

Negative scores for PC 3 would be linked to good early season vigor, perhaps an indication of winter hardiness and early weed competitiveness. Negative scores for PC 3 would also indicate greater biomass production per unit tissue N and earlier

maturity. This could be due to plant traits, but could also be due to lower soil inorganic N, since this component also had a strong positive loading for postharvest soil N. As soil N measurements in this experiment were not taken on individual plots, it is not possible to interpret lower postharvest inorganic soil N as a measure of plant efficiency, but instead it could be the cause of lower SPAD readings and earlier maturity. Greater biomass production per unit of tissue N is the traditional measure of NUE for perennial grasses in breeding programs and ecological studies (Berendse and Aerts, 1987), and could be very important for organic systems, especially in soft white winter wheat where high grain protein concentration is not desired.

In this experiment, genotypes in the conventional and control categories had the highest scores for PC 1. A mix of all categories had negative scores for PC 2, and 5K020007, 4J020275, 4J020187, 4J020274 and 4J020210-6 had the most negative scores for PC 3. These lines may have the best adaptation to organic systems in terms of NUE for these environments, and crosses between these lines could be used to combine useful traits for performance in organic systems. No genotypes had scores that correlated strongly to either location or year. Within categories, certain genotypes are closer to one location or the other, and this may be evidence of better adaptation to those environmental conditions. It is interesting that several of the organically bred lines had scores closer to the scores for the DeLongs location, since it has been certified organic much longer than Spillman, which is just completing the transition to organic certification.

5.4.2 Greenhouse Experiment

5.4.3 Annual genotypes

For the annual genotypes, the first PC explains more than twice the proportion of total variation as PC 2, and over 5 times as much as one original variable. PC 1 has positive loadings for %N at all plant stages, including both straw and grain %N, and positive loadings for anthesis, straw and grain total N. It has negative loadings for biomass traits other than grain weight, and a negative loading for 6leaf TN. Positive PC 1 scores would therefore indicate higher plant N concentrations and lower vegetative biomass but not lower yields.

PC 2 again has positive loadings for straw and grain %N, and positive loadings for both 6leaf and anthesis DAP. It has a fairly large negative loading on grain yield, even though the negative correlation between grain yield and %N is not strong. PC 3 is dominated by measurements on the 6leaf stage, with positive loadings on 6leaf DAP, biomass and TN. While the 6leaf stage may contribute to variation in the data, the lack of correlation between variables at 6leaf and those at maturity may limit the utility of these measurements.

The mean of the modern genotypes was positive for PC 1, and all modern genotypes had higher scores than the modern mean except Alpowa and Wawawai, which had negative scores below the mean of the historic genotypes. These two genotypes had problems with germination and survival, which probably affected the

results. The historic genotypes White Federation and Canus had PC 1 scores above the mean of the modern genotypes. For PC 2, the modern mean was negative and the historic mean was positive. Four modern genotypes and five historic genotypes had negative loadings below the modern mean, while only one modern genotype (Westbred Express) had a PC 2 score above the historic mean.

From the PC scores, it would appear that in general the modern genotypes had higher tissue N concentrations and lower vegetative biomass and that historic genotypes tended to have higher biomass production while yields were similar to the modern genotypes. This agrees with the ANOVA analysis in Chapter 4. Morris and Paulsen (1985) found that standard height varieties (mostly historic) had greater productivity under low N conditions than semi-dwarf varieties (mostly modern). The PC analysis is able to identify genotypes that fall outside of their respective categories, in this case White Federation and Canus fell closer to the modern category mean and Alpowa and Wawawai were more similar to historic genotypes in terms of N concentrations and biomass production, and Westbred Express was more similar to historic genotypes in terms of the rate of vegetative development (measured by DAP), grain and straw %N, and grain yield.

Addition genotypes

For the addition genotypes, the lines with positive scores on PC 1 are likely to have higher %N at anthesis and grain TN at maturity, but lower vegetative biomass

at all stages. CS+5E, CS+1E and CS+3E had the highest scores for this component, and *Th. elongatum* and AgCS had the lowest. For PC 2, 6leaf TN and biomass have a negative influence but anthesis TN, straw TN and straw %N all have positive influences. *Th. elongatum* had the highest score on this component and in contrast to PC 1, AgCS was not similar to *Th. elongatum* and was closer to CS+2E, which had the most negative score. Genotypes with positive PC 2 scores may not have good early growth, but have good vegetative biomass and N accumulation later in development. Loadings for traits related to grain at maturity are smaller for this component. Grain weight has a large positive influence on PC 3, as does grain TN, and grain %N has a large negative loading. This shows the negative correlation between grain protein concentration and grain weight and the positive correlation between grain weight and TN. *Th. elongatum* had the most negative score for this component, followed by AgCS, and CS+4E had the most positive score, followed by CS. This is of interest because CS+4E has a perennial growth habit, but groups near the annual parent for the component which is most influenced by grain traits, rather than with *Th. elongatum*, the perennial donor line.

Perennial genotypes

PC 1 is negatively influenced by both biomass and TN at 6leaf, and genotypes with high biomass and TN at this stage tended to have the most negative scores for PC1. Anthesis TN had a positive influence on PC1, so if genotypes had good

biomass and N accumulation at both stages, their scores tended to be closer to zero for this component. For PC2, anthesis biomass, straw weight and grain weight had positive factor loadings and anthesis %N, straw %N and grain %N all had negative factor loadings, again showing the negative correlation between grain protein concentration and grain weight. Lines with positive scores for this component were 03JP016, 03JP026, and 03JP004, and the most negative score was 03JP039. Crosses among these genotypes might provide useful new combinations of grain yield and protein traits. PC 3 had positive loadings for straw TN and %N as well as for 6leaf SPAD and DAP. Genotypes with positive scores for PC 3, particularly 03JP026 and 03JP036, may have good N uptake capacity. Straw TN and %N have positive correlations with grain %N so this may be important to grain protein content as well.

Comparison of perennial and annual genotypes

The joint analysis of perennial and annual genotypes did not show any overlap between growth habits for PC 1, only one annual genotype among the perennial lines for PC 2 and separate clustering patterns on the scatterplot. Perennials had positive scores for PC 1 and negative scores for PC 2, while annual genotypes had negative scores for PC 1 and positive to slightly negative scores for PC 2. It appears that perennials therefore had lower biomass and %N at the 6leaf stage, and higher straw TN, straw %N and grain %N based on PC 1. Based on PC 2, the perennials had lower anthesis biomass but higher anthesis %N, lower straw weight but higher straw

%N, and lower grain weight but higher grain %N than annuals. Genotype ranks on PC 3, 4, and 5 were more complex, with substantial overlap among historic, modern and perennial categories. The annual genotypes closest to perennials for PC 1 were Zak and Westbred Express, two modern varieties. Pacific Bluestem had a score close to the perennial mean for PC 2 and Arco was very similar to the perennial genotype with the least negative score for PC 2.

Correlations among measured variables in the greenhouse

While there were significant differences in factor loadings for the different measured variables among the three experiments and the joint analysis, there are some correlations that were present in most or all of the experiments. At 6leaf, biomass and TN were positively correlated. Traits measured at the 6leaf stage were not well correlated to those measured at maturity. In contrast, a study of maize genotypes sampled at 6-7 leaf stage found that leaf nitrate in young plants was positively correlated to grain yield and N uptake efficiency at low or high N levels and concluded that the N status of young plants may reflect the ability of a given genotype to absorb and store more or less N (Masclaux et al., 2001). We did not find such a correlation in this study among annual genotypes, chromosome addition lines or perennial wheat lines.

TN and %N at anthesis were positively correlated to straw TN and %N, and grain weight, TN and %N, although this correlation was stronger with the annual

experiment than with the addition or perennial experiments. Within sampling stages, DAP tended to show a positive correlation to biomass and TN. Biomass and %N generally showed a negative correlation within the vegetative stages. When comparing varieties at the same rate of N fertilizer, there is typically an inverse relationship between protein concentration and biomass, and in perennials the leaf N concentration is inversely related to dry matter and N yield (Davis et al., 1961; Kramer, 1979; Dubois and Fossati, 1981; May et al., 1991; Costa and Kronstad, 1994; Fowler, 2003; Wilkins et al., 1999). This was true in this study for the vegetative stages, however, at maturity there were only small negative correlations between straw weight and straw %N and between grain weight and grain %N.

5.4.4 Conclusion

Because of problems with multicollinearity and the effects of scale on the weight of variables in indices, PCA may be a useful tool in assessing complex traits such as NUE. PCA can be used to identify groups that were not immediately apparent in the original data (Wiley, 1981). The relationship among measured variables and among genotypes or treatments could be useful in assessing which measurements and locations are best in explaining variation among genotypes, and which genotypes have favorable combinations of traits or possess favorable characteristics that could be combined through crossing.

Because the principal components are not correlated, it is possible to interpret them independently. The total variation in the data is broken down into additive components (Wiley, 1981). When the PCs are easily interpreted as combinations of traits, genotype scores can be used to identify those genotypes with favorable combinations for crossing and selection purposes. Genotype scores can also be used to assess the amount of useful variation for these trait combinations. Even when PCs are not combinations of traits that would be helpful for selection, this type of analysis can determine the relationships among genotypes and among the original measured variables without the problems of multicollinearity found in multiple regression. Knowledge of genetic relationships can be used in preserving genetic diversity, for example among the historic varieties or within the perennial breeding program. Factor loadings and correlations for the measured variables can be used to determine the amount of redundancy in the data set and to eliminate variables which are either highly correlated to other variables or not well correlated to those variables of interest. In this case, the measurements taken at the 6leaf stage could probably be eliminated. SPAD meter readings, however, were not well correlated enough with %N at anthesis or traits assessed at maturity to replace any of these measured variables.

For a small study such as this, it is relatively easy to go through data by hand to look at combinations of traits and to compare genotypes for these combinations. In a larger study or breeding program where thousands of lines are planted each year, the ability of PCA to visually represent patterns among measured variables and

among genotypes could be very powerful. While PCA or any other statistical analysis cannot replace careful observation and selection, it may be a useful tool in identifying trends or genotypes that merit more detailed analysis and observation.

Acknowledgments

The authors would like to thank Steven Lyon, Kerry Balow and Meg Gollnick for extensive help in the management of this experiment. David Uberuagua, Margaret Davies and Ron Bolton provided technical expertise in the analysis of soil and plant samples. Richard Alldredge was of great help in the statistical analysis. This work was funded by the National Science Foundation under a Graduate Research Fellowship and by The Land Institute. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation or the Land Institute.

Bibliography

Berendse F., Aerts R., 1987. Nitrogen-use-efficiency: a biologically meaningful definition? Functional Ecology 1, 293–296.

Bertin P., Gallais A., 2000. Genetic variation for nitrogen use efficiency in a set of recombinant maize inbred lines I. Agrophysiological results. Maydica 45, 55–66.

Costa J.M., Kronstad W.E., 1994. Association of grain protein concentration and selected traits in hard red winter wheat populations in the Pacific Northwest. Crop Science 34, 1234–1239.

Davis W.H., Middleton G.K., Hebert T.T., 1961. Inheritance of protein, texture and yield in wheat. Crop Science 1, 235–238.

Desai R.M., Bhatia C., 1978. Nitrogen uptake and nitrogen harvest index in durum wheat cultivars varying in their grain protein concentration. Euphytica 27, 561–566.

Dhugga K.S., Waines J.G., 1989. Analysis of nitrogen accumulation and use in bread and durum wheat. Crop Science 29, 1232–1239.

Dubois J.B., Fossati A., 1981. Influence of nitrogen uptake and nitrogen partitioning efficiency on grain yield and grain protein concentration of twelve winter wheat genotypes (*Triticum aestivum* L.). Zeitschrift fur Pflansensuchtung=Journal of Plant Breeding 86, 41–49.

Fowler D.B., 2003. Crop nitrogen demand and grain protein concentration of spring and winter wheat. Agronomy Journal 95, 260–265.

Gáborčík N., 2003. Relationship between contents of chlorophyll (a+b)(SPAD values) and nitrogen of some temperate grasses. Photosynthetica 41, 2855–287.

Gauch H.G.J., 2006. Statistical analysis of yield trials by AMMI and GGE. Crop Science 46, 1488–1500.

Ghafoor A., Sharif A., Ahmad Z., Zahid M.A., Rabbani M.A., 2001. Genetic diversity in blackgram (*Vigna mungo* L. Hepper). Field Crops Research 69(2), 183–190.

Giunta F., Motzo R., Deidda M., 2002. Spad readings and associated leaf traits in durum wheat, barley and triticale cultivars. Euphytica 125, 197–205.

Kramer T., 1979. Environmental and genetic variation for protein content in winter wheat (*Triticum aestivum* L.). Euphytica 28, 209–218.

Lafitte H.R., Edmeades G.O., Taba S., 1997. Adaptive strategies identified among tropical maize landraces for nitrogen-limited environments. Field Crop Research 49, 187 – 204.

Lammer D., Cai X., Arterburn M., Chatelain J., Murray T., Jones S., 2004. A single chromosome addition from *Thinopyrum elongatum* confers a polycarpic, perennial habit to annual wheat. Journal of Experimental Botany 55(403), 1715–1720.

Lee W., Searcy S.W., Kataoka T., 1999. Assessing nitrogen stress in corn varieties of varying color. In: of Agricultural Engineers A.S. (ed.), Presented at the 1999 International ASAE Meeting. 2950 Niles Road, St. Joseph MI 49085-9659, pp. Paper No. 99–3034.

Loffler C.M., L.Rauch T., Busch R.H., 1985. Grain and plant protein relationships in hard red spring wheat. Crop Science 25, 521–524.

Ma B.L., Dwyer L.M., Fregeau-Reid J., Voldeng H.D., Dion Y., Nass H., 2004.

Graphic analysis of genotype, environment, nitrogen fertilizer, and their interactions on spring wheat yield. Agronomy Journal 96, 169–180.

Manly B.F.J., 1994. Multivariate Statistical Methods: A Primer, Chapman and Hall, London, chap. Principal Component Analysis. 2 edn., pp. 76–106.

Masclaux C., Quilleré I., Gallais A., Hirel B., 2001. The challenge of remobilisation in plant nitrogen economy. a survey of physio-agronomic and molecular approaches. Ann. Appl. Biol. 138, 69–81.

May L., Sanford D.A.V., MacKown C.T., Cornelius P.L., 1991. Genetic variation for nitrogen use in soft red x hard red winter wheat populations. Crop Science 31, 626–630.

Mohammadi S.A., Prasana B.M., 2003. Analysis of genetic diversity in crop plants – salient statistical tools and considerations. Crop Science 43, 1235–1248.

Morris C.F., Paulsen G.M., 1985. Development of hard winter wheat after anthesis as affected by nitrogen nutrition. Crop Science 25, 1007–1010.

Olfs H.W., Blankenau K., Brentrup F., Jasper J., Link A., Lammel J., 2005. Soil and plant based nitrogen-fertilizer recommendations in arable farming. Journal of Plant Nutrition and Soil Science 168, 414–431.

Oury F., Godin C., 2007. Yield and grain protein concentration in bread wheat: how to use the negative relationship between the two characters to identify favourable genotypes? Euphytica 157, 45–57.

Sinclair T.R., 1998. Historical changes in harvest index and crop nitrogen accumulation. Crop Science 38(3), 638–643.

Vidal I., Longeri L., Hétier J.M., 1999. Nitrogen uptake and chlorophyll meter measurements in spring wheat. Nutrient cycling in agroecosystems 55, 1–6.

Wiley E.O., 1981. Phylogenetics: The theory and practice of phylogenetics and systematics. John Wiley, New York.

Wilkins P.W., Allen D.K., Mytton L.R., 1999. Differences in the nitrogen use efficiency of perennial ryegrass varieties under simulated rotational grazing and their effects on nitrogen recovery and herbage nitrogen content. Grass and Forage Science 55, 69–76.

Wolfe D.W., Henderson D.W., Hsiao T.C., Alvino A., 1988. Interactive water and nitrogen effects on senescence of maize. II. photosynthetic decline and longevity of individual leaves. Agronomy Journal 80, 865–870.

Yan W., Hunt L., 2001. Interpretation of genotype x environment interaction for winter wheat yield in Ontario. Crop Science 41, 19–25.

Chapter 6

Decentralized selection and participatory approaches in plant breeding for low-input systems

Originally published as: Dawson J.C., Murphy K.M., Jones S.S., 2008. Decentralized selection and participatory approaches in plant breeding for low-input systems. Euphytica 160: 143-154. DOI 10.1007/s10681-007-9533-0.

6.1 Abstract

Heterogeneous environments make it difficult to apply consistent selection pressure because often it is difficult to identify a single or a few superior genotypes across all sets of conditions. However, when the target system is characterized by heterogeneity of environmental stress, varieties developed in high-yielding conditions may fail to satisfy farmers' needs. Although this type of system is often found in

marginal environments of developing countries, heterogeneous environmental conditions are also a feature of organic and low-external-input systems in developed countries. To meet the needs of these systems, breeding programs must decentralize selection, and although decentralized selection can be done in formal breeding programs, it is more efficient to involve farmers in the selection and testing of early generation materials. Breeding within these target systems is challenging, both genetically and logistically, but can identify varieties that are adapted to farming systems in marginal environments or that use very few external inputs. A great deal has been published in recent years on the need for local adaptation and participatory plant breeding; this article reviews and synthesizes that literature.

Keywords: participatory plant breeding; organic agriculture; heterogeneous environments; on-farm selection; genotype by environment interactions.

Abbreviations: PPB Participatory Plant Breeding; CIMMYT International Maize and Wheat Improvement Center; GxE genotype by environment interactions; ICARDA; International Center for Agricultural Research in Dry Areas.

6.2 Plant breeding for low-input systems

Plant varieties adapted to low-input systems are needed in both developed and developing countries. Organic or low-external-input systems in developed countries may resemble farming systems in marginal environments of developing countries

because environmental stress is heterogeneous, there are few varieties that meet the diverse needs of farmers in such systems and there is very little interest from the commercial seed sector (Desclaux, 2005). Improving varietal performance in such systems can help improve farmers' livelihoods in all parts of the world. In developing countries, access to inputs is often limited or non-existent, and farmers need varieties that will perform well when grown under severe stress. In developed countries, inputs are usually available, but many farmers want to reduce there use for economic or environmental reasons. Reducing the need for off-farm inputs increases commercial farmers' profit margin and subsistence farmers' food security. In addition, the total use of agricultural chemicals, particularly nitrogen (N) fertilizer, will need to be significantly lowered if agriculture is to be sustainable.

It is often more difficult to identify superior genotypes or to apply consistent selection pressure under low-input conditions because of environmental heterogeneity (Haugerud and Collinson, 1990). When moving from high to low yielding environments, the genetic variance generally decreases while the error variance may increase (Bänziger and Cooper, 2001; Bertin and Gallais, 2000; Ud-Din et al., 2004; Brancourt-Hulmel et al., 2005). Because the error variance does not usually decrease as much as the genetic variance, experiments in low-yielding conditions may have a lower chance of detecting a statistically significant difference among lines (Bänziger et al., 1999). In cases where soil fertility is low, variability in nutrient supply has a large impact on crop performance. In other cases, low-input systems may have high

soil fertility and high yields due to the use of crop rotations, green manures and animal manures. These systems are often called low-external-input systems, and are much more complex in terms of nutrient cycling than conventional agricultural systems, so there is likely to be a good deal of variability in the nutrient supply over space and time. Variability in soil characteristics or nutrient availability complicates experimental design and analysis, but it is possible to overcome these obstacles and achieve genetic gains when breeding crops for low-input systems.

6.2.1 Heritability and genotype by environment interactions

Because of the decrease in genetic variance in low-input environments, many breeders prefer to conduct selection in relatively high-input environments, assuming the genetic gains will carry over to low-input conditions. Selecting in favorable environments for performance in marginal environments is a type of indirect selection, and is justified if the heritability of traits is significantly higher in high-yielding environments (Ceccarelli, 1994). The efficiency of indirect selection depends not only on the heritability, but also on the genetic correlation coefficient between the two environments. High genetic correlation coefficients between two environments makes crossover GxE interactions less likely because the environments are similar, but a low genetic correlation coefficient means that the lines that do best in each environment will probably be different. The efficiency of selecting in environment x for performance in environment y is given by the equation:

$CR_x/R_x = r_g * h_y/h_x$ where CR_x is the correlated response in y to selection in x, R_x is the response to selection in x, r_g is the genetic correlation coefficient, h_y and h_x are the square roots of heritability in y and x respectively (Ceccarelli, 1994).

With a low genetic correlation coefficient, heritability in environment x must be several times larger than the heritability in y for indirect selection to be useful. Also, if the genetic correlation coefficient is negative, the heritability is no longer relevant, as indirect selection will be counterproductive (Ceccarelli, 1994). Brancourt-Hulmel et al. (2005) showed that indirect selection in maize under high N for performance at low N became increasingly inefficient as the N stress increased. Similarly, a study of European maize lines showed that the genetic correlation coefficient decreased and became negative as N stress increased (Presterl et al., 2003). In ICARDA's barley breeding program Ceccarelli (1994) calculated that it was 28 times more effective to conduct direct selection under farmers conditions with local germplasm than to conduct selection in a high-yielding environment with introduced germplasm.

A review of the experimental evidence shows that heritability is not intrinsically lower in low-input or marginal environments (Ceccarelli, 1994). For example, in CIMMYT maize lines, Agrama et al. (1999) found equal heritability estimates at high and low N for traits related to nitrogen use efficiency (NUE). Most studies showing that heritability is lower in low-input environments use genotypes originally selected in favorable environments, which are then tested in low-input environments. In a series of environments with progressively higher stress levels,

there will be a point at which two genotypes change rank in performance. At the crossover point, the heritabilities will be lowest, as it is difficult to discriminate between genotypes. At the high and low ends of the spectrum, heritabilities will be higher, so if the target environment is above the crossover point, selection in a high yielding environment will produce the best results. If the target environment is below the crossover point, selection in a low-yielding stress environment will be best (Ceccarelli, 1996a). When crossover interaction occurs, the material from high-input selection will be poorly adapted to the low input conditions and calculations of heritability will be low (Ceccarelli, 1994).

GxE interactions become more important as selection environment and target environment diverge (Ceccarelli and Grando, 1999), so selection for specific adaptation becomes more important as yield differences between high and low input environments increase (Bänziger and Lafitte, 1997). Differences in system management, such as organic or conventional practices, can also result in crossover GxE interactions, and lines selected in one system and grown in another will not be as optimal as lines selected in the target system (Murphy et al., 2007). These crossover GxE interactions can be exploited by breeding for superior adaptation within the target environment instead of looking for high average yields across locations and years. Selection for specific environments involves a positive interpretation of GxE interaction, where top performing lines are selected in each target environment. A negative interpretation is more common, where GxE

interaction is seen as a barrier to achieving broad adaptation, and top performing lines in particular environments may be thrown out in favor of those that have the best average performance across environments (Ceccarelli, 1996b). Sometimes lines can perform well under both high and low-input conditions in breeder-managed trials, but do not outperform local varieties in farmers fields (Berg, 1997).

6.2.2 Broad vs. specific adaptation and stress tolerance

Varieties that are thought to have broad adaptation across environments may in fact be narrowly adapted to environments that can be modified to be more similar to research station conditions through the use of fertilizers and other inputs. This type of variety has been mostly adopted in favorable environments, while in many marginal areas, there is limited use of modern varieties (Ceccarelli, 1994). This could be due to lack of access to seed, but even in regions where modern varieties have been partially adopted, landraces are still grown. There are many cases where landraces still yield better than modern varieties in farmers' fields (Ceccarelli and Grando, 1999). Applications of fertilizer may be considered too risky in marginal environments where environmental constraints such as drought severely limit crop yields or cause crop failures in many years (Ceccarelli, 1994). Marginal environments include areas where environmental and socio-economic conditions result in complex stresses and high risks to agricultural production. Most of these areas are too different from more favorable production areas to benefit, even indirectly, from

breeding in high input systems (Almekinders and Elings, 2001).

Many farmers are most interested in minimizing the amount of variation they observe over years, that is, they prefer yield stability over time rather than high potential yield in favorable years. In terms of meeting social and economic needs, breeding for stability and minimizing crop failures is probably the most important breeding objective (Ceccarelli, 1994). This is true in developed as well as developing countries. For example, the wheat breeding programs at Washington State University work with farmers in the driest areas (200-300 mm precipitation) of Eastern Washington who would prefer a variety that yielded 40 bushels per acre ($2.7\,\mathrm{tons\,ha^{-1}}$) every year. They would be willing to give up the few years where they get 60 bushels ($4\,\mathrm{tons\,ha^{-1}}$) to avoid the years with 20 bushels ($1.3\,\mathrm{tons\,ha^{-1}}$) or less.

Temporal variation can be minimized by breeding heterogeneous populations similar to landraces that have specific adaptation to the target environment (Ceccarelli et al., 2001). In a study of barley breeding, groups of genotypes selected in stressful environments generally had lower slopes and coefficients of variation in regression analyses than groups selected in high-input environments, indicating better stability across the range of locations tested (Ceccarelli, 1994). Genetic diversity for resistance and stress tolerance buffers against abiotic and biotic stresses which may change from year to year, giving more stability to the population as a whole, even without significant variation for agronomic traits such as quality or maturity (Ceccarelli, 1994; Sthapit and Jarvis, 1999; Witcombe et al., 1996). Farmer

bred varieties often have large amounts of allelic variation within the variety, and farmers may grow multiple varieties within a field, which helps to reduce phenotypic variation under stress (Cleveland et al., 1999). Breeders, however, often try to minimize the amount of variation over space by breeding for broad adaptation (Ceccarelli and Grando, 1999). The focus on selecting for broad adaptation has replaced selection for stability over time as modern breeding replaced seed selection on farms (Riley, 2003). Involving farmers in the selection process with breeders in the formal sector tends to maintain more diversity in the region because farmers look for genotypes with good temporal stability while breeders tend to focus on broader adaptation (Ceccarelli et al., 2001).

It is possible that the traits required by farmers in low-input environments are too diverse to be fully addressed by a centralized breeding program, even one focused on agriculture in marginal areas (Smith et al., 2001). Many breeders may not be aware of the wide range of traits farmers working with such systems desire (Desclaux, 2005). For example, in observing the characteristics desired by farmers in very dry production environments, breeders learned to select for straw productivity and grain filling ability under multiple stresses. Low input, drought conditions cause a reduction in plant height, so the best lines in low input conditions were tall plants with soft straw when grown in high-input conditions. When faced with the stress conditions, these genotypes become short and stiffer, so that lodging is not a problem, but the straw is still palatable to livestock. Superior genotypes in these

conditions would be the opposite of what modern breeding programs would look for in a high-potential environment (Ceccarelli, 1996a). The fact that some key traits for low-input conditions are not apparent until selection and evaluation is done in those environments is a strong argument for conducting breeding programs in the target environment.

Selection for tolerance to stress may reduce yields when grown in favorable conditions compared with cultivars selected in the favorable environments. High grain yield in very divergent environments appears to be controlled by different sets of alleles across many loci (Bänziger and Lafitte, 1997; Ceccarelli, 1994). Therefore, varieties with adaptation to severe stress are less likely to be selected when tested in high-input environments (Ceccarelli, 1996a). Data on the utility of using both stressful and non-stressful environments for selection is contradictory. Progress made by alternating breeding nurseries between low and high-input environments may depend on the breeding strategy. It appears that such alternation is successful with pedigree breeding methods but detrimental with a bulk breeding strategy (van Ginkel et al., 2001). Since environmental conditions in low-input systems rarely approach the conditions on high-input research stations, the potentially lower yields of lines selected in high stress conditions when grown under optimal conditions is unlikely to be a problem (Ceccarelli, 1996a).

When stress factors vary over seasons, selecting in different nurseries may help subject breeding lines to the multiple stresses that they could face in farmers' fields.

An index of selection that weights performance in multiple environments may be helpful in selecting for stressful conditions. Ud-Din et al. (2004) found that weighting performance in irrigated and drought stressed environments allowed for faster progress under dryland conditions than direct selection under drought stress. This occurred even though the genetic correlation coefficient between the two environments was not significantly different from zero. However, alternating selection in high and low-yield potential environments could be ineffective because only lines that do well in both are selected, rather than lines that may do very well in one of the environments but not in the other (Ceccarelli, 1994).

If certain environmental factors predictably limit yield, but are not always observed in farmers fields, managed stress nurseries may be useful (Atlin et al., 2001; Wade et al., 1996; Cooper et al., 1995, 1996). An advantage of these nurseries is that thousands of lines can be evaluated at once for their response to a specific stress (Bänziger et al., 1999). Nurseries with human-created stress factors, such as pathogen pressure, are useful for screening for disease resistance, but it is not clear whether they are efficient for abiotic stresses (Basford and Cooper, 1998). In an Australian wheat breeding study, there was generally a good correlation between performance in managed stress nurseries and on-farm, but some lines which did well in the managed stress nurseries did poorly when grown on-farm (Cooper et al., 1995, 1996) Because low-input environments generally have multiple interacting environmental stress factors, designing managed stress environments that capture

the key elements limiting yield may be difficult. It is challenging for both farmers and breeders to predict the likelihood of certain types of interactions (Bänziger et al., 1999), so the best way to guarantee breeding progress is to consistently work in the target environment. This means decentralizing the selection environments of a breeding program to include nurseries in all target environments.

6.3 Decentralized selection and participatory plant breeding

Decentralization of selection environments is critical to achieve good adaptation to marginal agricultural environments. Although decentralized selection and participatory plant breeding (PPB) are separate ideas, in practice it is difficult to separate the two (Ceccarelli et al., 2001). Having broadly adapted varieties justifies salaries and research expenses in a centralized system (Smith and Weltzien, 2000). If breeding is to be decentralized, the same amount of resources are not available for each location. By enlisting the support and expertise of farmers, decentralized selection becomes possible. PPB is usually focused on making productivity gains in marginal areas and non-commercial crops, enhancing biodiversity and the conservation of genetic resources, developing germplasm for socially or economically disadvantaged groups and making breeding programs more cost effective through decentralization (Sperling et al., 2001). This is because of the contribution of farmers

in terms of management and because their expertise helps ensure that breeding effort is not wasted on lines that are never adopted.

Farmers involved in PPB are researchers alongside the plant breeders. They set priorities for the breeding process, make crosses, screen germplasm, test selections in multiple environments and lead the seed multiplication and distribution process (Sperling et al., 2001). Certain farmers are known for their skill in seed selection and saving and are especially good to have on a participatory breeding team (Smith and Weltzien, 2000). Working with a few enthusiastic and well trained farmers may improve the efficiency of a participatory breeding program as farmer experts can make selections for their entire community and spread the benefits of participatory plant breeding through seed exchanges or community plots (Gyawali et al., 2007; Sperling et al., 1993).

While the skill of farmers in selection and their ability to handle distinct populations is often questioned, in many projects farmers have proved to be extremely competent. In Syria, farmers were more effective than breeders at selecting superior barley genotypes in their own fields, and farmers were able to handle large numbers of entries, including segregating materials in early generations (Ceccarelli et al., 2001). Selection on-farm, using germplasm from local landraces, produced pure lines that out-yielded the landraces by 20% in farmers' fields (Ceccarelli, 1996a). This was a productive short term strategy for improving yields in stressed environments, and these superior genotypes may eventually be used in

crosses or blended to form heterogeneous improved landraces. It is important to consider the impact of selecting homogeneous lines from landraces on genetic diversity, and to have a long-term breeding strategy that maintains genetic diversity since this diversity is one of the primary reasons that landraces have yield stability (Ceccarelli, 1996a). In the PPB program at ICARDA, farmer skills increased over several seasons, and they became active participants in suggesting new crosses and selection criteria. Farmers were enthusiastic about the potential of making selections from landraces and demanded that the program be extended to other crops in addition to barley (Ceccarelli et al., 2001).

Similarly, in a participatory rice breeding program in Nepal, farmers increased the effort and time they invested in breeding as the project started showing results (Sthapit et al., 1996). Joint selection by farmers and breeders have produced most of the successful lines from this program. A simple bulk breeding strategy is used, with bulk populations created by breeders and then grown in large populations by interested farmers. Lines selected by farmers have become popular and are spreading to other villages in the area (Gyawali et al., 2007).

In Rwanda, farmers identified as bean experts helped make selections on station by ranking breeding lines for traits of interest and then taking 2-3 of these lines to grow in home gardens alongside their traditional mixtures. The lines identified by local farmers out-yielded the local mixtures 64-89% of the time, with an average increase in yield of 38%. In contrast, breeder selections out-yielded local

mixtures 41-51% of the time on a national scale, with an average 8% increase in yield (Sperling et al., 1993). Six seasons later, 71% of the farmer selected varieties were still being grown; 32% were used to create new mixtures and 35% were incorporated into existing mixtures of farmer varieties. One of the most popular varieties from the formal sector had a 61% chance of still being grown six seasons later (Sperling et al., 1993). The farmers were aware of GxE interactions and were fairly accurate at predicting how certain lines would perform based on their observations on the research station. Sperling et al. (1993) found that by working with farmers, promising lines were selected earlier, more lines were selected and these varieties were better adapted to local conditions as shown by higher yields on-farm.

6.3.1 On-farm selection

(Atlin et al., 2001) proposed three main strategies to improve on-farm selection: increasing selection intensity by using larger populations, increasing the genetic correlation between the target and selection environment by making sure the selection environment is highly representative of the target population of environments, and increasing the heritability of the traits of interest by improving the precision with which genotypes are evaluated. Further work is still needed to improve the precision of on-farm trials in highly variable environments (Ceccarelli et al., 2001).

Farmers often make selections after harvest, which excludes selection on plant traits such as decreased barrenness and improved stay-green characteristics under drought stress. For example, in a survey of Ecuadorian farmers, over 90% selected seeds for the next season after harvest based on ear and kernel appearance, without considering plant traits in the field (Almekinders et al., 2007). Field stratification and gridded selection where farmers select a certain percentage of plants and ears from each part of the field avoids this problem and increases gains from selection (Smith et al., 2001). Improved experimental designs, appropriate for farmer's conditions, can make it possible for farmers to achieve greater response to selection. These designs increase the ability of farmers to make selections based on genotypic differences without using complex statistical models (Bänziger et al., 1999; Cleveland et al., 1999). This includes training in methods of selection for correlated traits such as index selection (Riley, 2003).

It is often difficult to get enough seed to distribute to several farmers for participatory selection from early segregating generations. Farmers can help to select promising line on-station in early generations, then selection can move to farmers' fields when enough seed is available (Witcombe et al., 1996). Networks of farmers evaluating the same lines could serve as replicates in a multilocational trial. With more locations, it is possible to identify promising entries in earlier generations when seed supplies are still limited (Witcombe et al., 2005b). Genotype by year by location interactions are often the largest component of variation, and this is best

dealt with by replicating over locations and years (Atlin et al., 2001). Because of large genotype by year by location interactions, programs that combine the results of several farmers are more likely to be effective than selections by individual farmers (Bänziger et al., 1999). As the genes desired occur with greater frequency in the population, the phenotypic variance decreases, so visual selection is less effective and more replications are necessary. Products that come from programs with adequate replication and selection intensity tend to perform well across areas with similar environmental conditions (Atlin et al., 2001).

Formal breeding programs usually make many crosses, and only advance a small population of progeny from each cross for selection in later generations. In PPB, a more efficient strategy may be to carefully choose parents based on important characteristics, make a few crosses with these parents, and then increase the progeny population size for on-farm selection (Witcombe and Virk, 2001). An unadapted parent might be used from a breeding program in another region or in a high-input system which has good disease and pest resistance, good quality or high yield potential, but most of the parental germplasm should posses good adaptation to the target environment. In this way PPB can benefit from existing formal breeding programs and the potential wide adaptation of their products (Witcombe and Virk, 2001), as well as the specific adaptation of landraces or varieties popular with local farmers.

Suneson (1956) proposed an evolutionary breeding method where diverse

parent material was crossed and the resulting population was allowed to evolve through natural selection in cropping environments. Although initial yields were very low, fifteen cycles of natural selection produced a population that was fairly high yielding, with excellent yield stability and disease resistance. Improvements in yield related traits were most apparent in populations that were always grown either in favorable environments or in unfavorable environments so that directional natural selection was consistent (Allard, 1999). Suneson stated that this method would produce new varieties at minimum cost with assurance of adaptability, and could be used to develop either superior populations or pure lines, through selection of individuals out of the population. The primary drawbacks to this method are the length of time required due to low selection intensity, and the inability to select for quality traits that do not confer a fitness advantage. Combined natural and artificial selection within a local environment may be a highly effective selection method, combining evolutionary and directional selection strategies (Murphy et al., 2005).

High selection intensity can be achieved through mass selection with large populations (Atlin et al., 2001). Farmers can use mass selection by walking through a population and removing plants they do not like, and/or selecting superior individuals and bulking the seed from these for the next generation. In a self-pollinated population, using multiple parents with diverse genetic backgrounds would increase the amount of genetic variation within the population. After several generations, individual plants in a bulk population would reach homozygosity, but

the population would still be heterogeneous. Farmers could then select individual plants and produce pure lines of superior genotypes. The most successful pure lines could be bulked and grown as a blend, which would meet end-use marketing standards but still be capable of adaptation (Murphy et al., 2005). The choice of high end-use quality parents is particularly important for this method, as quality is difficult for farmers to asses if they grow a crop for the commercial market, and is not necessarily improved by natural selection (Murphy et al., 2005).

Some breeders claim that participatory breeding projects involve too much risk for farmers, however, farmers often have sophisticated risk management systems. Farmers use genetic variation to reduce their risks, planting both multiple varieties of the same crop and several different crops. They usually try new material on their worst land, thus any new variety first must grow in the poorest conditions. If a variety does well in the most marginal spot, it may be planted on more productive land. This contrasts with the tendency of researchers to put experimental plots on the best and most uniform ground (Sthapit et al., 1996). There is a greater risk that, through breeding for high-input systems, formal breeding programs will produce varieties that are seldom adopted because they do not work in marginal farming areas (Witcombe, 1996). This has occurred in many areas because landraces either out-yield or have greater stability than modern varieties released by formal breeding programs. However, because farmers may exchange seeds frequently, and often do not have consistent strategies for selection, landrace germplasm may not have been

subjected to continuous directional selection. A more conscious effort is needed to make full use of local knowledge and germplasm (Berg, 1997).

An integrated system of plant breeding could use aspects of both formal and participatory breeding. Plant breeders would enhance useful germplasm, both from local landraces and promising introductions. Local communities do not always have access to the resources preserved in germplasm collections and genebanks, especially in developing countries. Establishing partnerships with plant breeders at public institutions is a potential mechanism for returning this germplasm to farm communities, and for making use of germplasm that has useful traits but is not local (Berg, 1997). It would be more useful if this germplasm was first crossed to local materials, making enhanced populations which would then be released to farmers and selected on farm (Berg, 1997). Selection and evaluation would be done with or by farmers, with continued exchange of information and ideas (Riley, 2003). On-station screening is still important in participatory projects, particularly for disease resistance and for traits which are difficult for farmers to assess (Smith and Weltzien, 2000; Witcombe, 1996). Breeders contribute their knowledge of genetics and statistics and farmers contribute their knowledge of the specific challenges of their farming system and of plant traits needed to overcome these challenges. When landraces are used as parents along with more modern varieties and there is maximal farmer input, the breeding strategy can complement *in situ* conservation by conserving favorable alleles in landraces that have been selected in that particular

environment. PPB conserves and creates genetic resources in farmers' fields (Witcombe et al., 1996). It also increases the efficiency of selection by raising farmer's awareness and knowledge of genetic processes.

6.3.2 Participatory plant breeding in high-input environments

The relevance of participatory plant breeding to developed agricultural systems is often questioned. In such systems, the use of high-yielding modern varieties is the norm, and little if any of the farm output is for the farmers' own consumption. The use of off-farm inputs such as fertilizer and pesticides makes the growing conditions similar from farm to farm and region to region, so a few varieties may perform well over a wide spectrum of environmental conditions. However, there is concern over the increasing cost of inputs and growing interest in precision farming and sustainable agriculture. Organic and low-external-input farmers choose to limit their inputs and rely on biological processes for many reasons, including economic and environmental concerns. A growing number of these farmers are interested in participatory approaches to plant breeding (Desclaux and Hédont, 2006). Highly productive areas have the potential for greater diversity in crop species and varietal diversity within species (Witcombe, 1999). Breeding crops adapted to specific farming systems and ecological zones is important for these systems, and will require

decentralized breeding programs that can address the needs of a diverse landscape.

For this to be successful, it is essential to have farmers actively participating in the research process. Farmer participation can take many forms, from helping to set research priorities and breeding goals, to selecting from diverse plant populations on their farms, to evaluating nearly finished varieties and giving feedback on varieties that have been released. Farmers in developed countries are as diverse in their interests and needs as farmers in developing countries, and there should be options for involvement at all stages of the breeding process. Many PPB projects are initiated in intermediate stress zones, and there are also examples of projects in low-stress environments where end-user preferences are fairly well defined. This is often to help farmers gain greater control of their seed supply, or to expand varietal diversity in areas which are predominantly monocultures (Sperling et al., 2001)

Farmers in industrialized agriculture rely largely on the private sector for the seed they plant each year, and to a lesser extent on public plant breeding programs. Wheat is one of the few species where the public sector is still the major source of new varieties. The process of relinquishing control of the seed supply began in the early twentieth century with the advent of hybrid corn. The process of creating hybrid corn is relatively simple, but the vast number of crosses and the record keeping required to keep track of them shut most farmers out of the process. After the professional field of plant breeding began to develop, breeders only worked with farmers if they needed more land for nurseries, and questioned whether farmers were

capable of making crosses and keeping track of progeny lines (Fitzgerald, 1993). Seed companies also pressured the USDA to stop encouraging farmers to save seed, claiming that farmers did not have the knowledge to save high quality seed or to work on breeding their own varieties (Fitzgerald, 1993). Both traditional agricultural practices and modern participatory plant breeding projects have shown otherwise.

The assumption is that formal plant breeding programs serve high-yielding environments well, because many of the environmental risks and constraints of marginal environments are absent in more favorable environments or can be overcome with the application of agrochemicals such as fertilizers (Witcombe, 1999). Many modern agricultural systems in high-yielding areas have adopted a monoculture of one or a few crops. This is often to simplify management and to increase profitability, both for farmers and breeding companies. Larger breeding programs can invest in larger testing nurseries and small-scale programs have limited ability to compete. Smaller testing networks have lower power to detect superior lines, so small-scale breeding programs have difficulty staying in business. This results in the consolidation of breeding programs and an economic incentive to release fewer, broadly adapted lines (Atlin et al., 2001). However, because of economic forces such as increased costs of inputs, including seeds, and stagnant or falling crop prices, many farmers are looking for alternatives to the commodity system. Diversity can provide buffering capacity for the system and for farm incomes, so many farmers are now looking to re-diversify and grow a range of higher-value products. For farmers

growing for the commercial market, it is important to also involve processors and end-users in the process, and the development and distribution of varieties through PPB should be linked with market opportunities (Almekinders et al., 2007).

Some public sector programs are already highly participatory because they are funded by commodity commissions where farmers fund the research projects they feel are most relevant (Witcombe et al., 2005b). Breeders usually use varieties that have been widely adopted by farmers as parents in formal breeding programs, which is an established feedback mechanism where the popularity of a variety indicates farmer preference for that combination of traits (Witcombe et al., 2005b). However, in under-served environments, farmers may not have access to varieties that truly meet their needs and therefore they grow varieties that are not ideal. Using these varieties as parents might not address the true needs of the farmers growing them. In general, participatory plant breeding is most useful where meeting end-user quality concerns is challenging. In high productivity environments, the risk of a mismatch between environmental conditions on the breeding station and those in farmers field is less, but still exists when breeders use the recommended "best agronomic practices" which may not be feasible for farmers due to economic cost or other constraints (Witcombe et al., 2005b).

6.3.3 Distribution of varieties from participatory plant breeding

Even though most PPB projects are based on single farms or small communities of farmers, the resulting varieties may be useful to a much larger group of farmers who have similar environmental conditions on their farms. Although the varieties developed through PPB will have specific adaptation to certain environmental conditions, it is likely that they will also perform well on farms that share similar climates and soil types. It is unlikely that they will spread as far as varieties specifically targeted to have wide adaptation in higher input systems (Morris and Bellon, 2004), but it is possible that they will benefit many farmers in neighboring areas. Genetically variable materials such as multi-lines, mixtures, open pollinated varieties and synthetics make it more likely that they will be useful to farmers in environments that differ from the original selection environment (Smith et al., 2001). This is because the existing genetic diversity in these materials buffers performance when exposed to new environmental conditions and in the case of out-crossing they can continue to evolve. Farmers may distribute heterogeneous materials through the informal seed sector and these can continue to diversify and evolve (Berg, 1997).

Local germplasm is still the primary, and sometimes the only, source of seed in developing countries (Almekinders and Elings, 2001). Strengthening the seed exchange system and helping farmers distribute disease and weed-free seeds helps to

make the products of plant breeding more widely available (Riley, 2003). Establishing links with local NGOs or farm groups that know how best to distribute seed can help with more widespread distribution of a promising variety to farmers who have similar environmental constraints and production systems (Witcombe, 1996). Using the informal seed sector and PPB instead of a formal approach to variety testing and release may put the products of plant breeding into farmers fields five to six years earlier. The informal seed sector can work equally well in high-input environments, as there is usually extra seed farmers can distribute (Witcombe, 1999).

Formally releasing a variety can make the results of PPB available to many farmers outside the immediate area in which it was developed. If it were possible to release heterogeneous varieties, i.e. modern landraces, through the formal seed sector, the benefits of PPB could have an even greater impact. Unfortunately, many developing countries have variety release requirements similar to those in developed countries, which are designed to release a few widely adapted cultivars for intensive agricultural systems that can be made uniform through management practices (Witcombe, 1996). This is often not appropriate for the diverse cropping systems and environmental stresses found in low-input agriculture, in either developed or developing countries. Genetic uniformity is usually not demanded by farmers, although the variety release process may require it.

For the formal varietal release process to work for participatory plant breeding, data on farmer perceptions and demand for seed need to be considered by varietal

release committees, rather than almost total reliance on yield data from scientifically managed trials (Witcombe et al., 1996). Authorities may not feel that data based on farmer-managed trials is as precise or relevant as data produced on research stations, however, projects involving farmer assessment of varieties shows remarkable consistency in farmer rankings. In an example of participatory selection of rice in India, farmers ranked varieties similarly, even for traits such as tillering and panicle length that are harder to measure than yield, maturity and height. This information was more relevant than the multi-locational trial data which was primarily measuring yield and had significant GxE interaction (Joshi and Witcombe, 1996). Farmers agreed with each other and breeders on superior varieties, probably because farmers selected to participate had good seed selection skills, and breeders were aware of what traits were important to farmers (Sthapit et al., 1996).

If superior varieties are identified through PPB that are suitable for similar low-input farming systems and environments across a broader geographic range, intellectual property rights (IPR) may become an issue (Smith et al., 2001). The exchange of varieties between countries is often restricted because of the belief that intellectual property rights must be defended, but in most cases there are no plant breeders' rights in the countries involved (Joshi and Witcombe, 1996). Farmers do not receive any royalties, although they do receive an indirect benefit through investment in further research and breeding and by recognition of the role of farmers in germplasm conservation and improvement (Sthapit and Jarvis, 1999). The

improved varieties themselves are generally the most useful compensation to the farmers. In some cases, it is possible to compensate farmers for their time, and to purchase the seed grown for the breeding program if the farmer does not want to keep it. Most public sector plant breeders do not get royalties or any financial gains from the development of their varieties, so there are no profits to be shared. PPB schemes would be problematic for private companies, because profits would need to be divided, and companies might worry about competitors taking varieties from fields if they were freely distributed. This is a major reason why public sector plant breeding programs are vitally important in underserved and marginal areas (Witcombe, 1996).

6.3.4 Scientific relevance of participatory plant breeding

The perception exists that farmer participation in research interferes with objectivity, precision, control and repeatability of experiments so participatory methods may not generate predictive theories (van de Fliert and Braun, 2002). This perception discourages researchers from using participatory methods, even if examples of successful participatory projects exist (Morris and Bellon, 2004). Reasons for not including farmers are often based on the assumptions that breeders have training that gives them an advantage in conducting selection and that complex systems of selection and thousands of entries are needed, which farmers are not equipped to handle (Witcombe et al., 2005b). Breeders may also feel that they require special training in participatory breeding methods, and such training is not

usually part of a plant breeding training program (Morris and Bellon, 2004). Using farmers' practices may complicate the experimental design and analysis (Haugerud and Collinson, 1990).

However, breeders and farmers have complementary skills that can contribute equally to successful varietal development in complex environments. Breeders have training in selection theory and experimental design; farmers have valuable knowledge about environmental conditions, the performance of varieties on different parts of their farm and the characteristics that make a variety successful in their region. Participatory research does not have to compromise the scientific contribution of research when appropriate experimental designs and selection strategies are used. The choice of strategy depends on the logistical capabilities and end goals of both the breeding programs and the farmers. Many farmers already do their own kind of research in testing and adapting new ideas and technologies (Conroy et al., 1999). Involving farmers in the selection phase of plant breeding is not always essential, but in certain situations it becomes critical to have farmer input during selection. These situations include those where farmers trade-off multiple traits against each other and if desirable end user qualities cannot easily be assessed with laboratory methods (Witcombe et al., 2005a).

6.4 Conclusion

The need to reduce external inputs in agricultural systems throughout the world is a challenge for both plant breeders and farmers. Including farmers in the research and breeding process will help to meet this challenge by developing varieties that are well suited to particular cropping systems and environments. Participatory plant breeding can benefit farmers in marginal environments in both developed and developing countries, and also those farmers who are seeking to lower their synthetic inputs for environmental or economic reasons. Because low-input systems are highly heterogeneous, there will need to be decentralization of the breeding process for it to be successful. The most efficient way to decentralize selection is to have breeding nurseries or populations on farms in the target environment, and to recruit interested farmers to help set priorities, evaluate breeding lines or select promising types in early generations. While these methods do not compromise scientific integrity, it will take a shift in priorities and perspectives at many institutions. Many researchers in developing countries are already doing participatory research, but much more can be done to reach the full potential for this research in developed agricultural systems.

Acknowledgments

The authors would like to thank two anonymous reviewers for their comments. This material is based upon work supported by the National Science Foundation

under a Graduate Research Fellowship. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

Bibliography

Agrama H.A.S., Zakaria A.G., Said F.B., Tuinstra M., 1999. Identification of quantitative trait loci for nitrogen use efficiency in maize. Molecular Breeding 5, 187–195.

Allard R.W., 1999. Principles of Plant Breeding. John Wiley and Sons, Inc., New York. Second Edition.

Almekinders C.J.M., Elings A., 2001. Collaboration of farmers and breeders: Participatory crop improvement in perspective. Euphytica 122, 425–438.

Almekinders C.J.M., Thiele G., Danial D.L., 2007. Can cultivars from participatory plant breeding improve seed provision to small-scale farmers? Euphytica 153, 363–372.

Atlin G.N., Cooper M., Bjørnstad Å., 2001. A comparison of formal and participatory breeding approaches using selection theory. Euphytica 122(3), 463–475.

Bänziger M., Cooper M., 2001. Breeding for low-input conditions and consequences

for participatory plant breeding: Examples from tropical maize and wheat. Euphytica 122(3), 503–519.

Bänziger M., Edmeades G.O., Lafitte H.R., 1999. Selection for drought tolerance increases maize yields across a range of nitrogen levels. Crop Science 39, 1035–1040.

Bänziger M., Lafitte H.R., 1997. Efficiency of secondary traits for improving maize for low-nitrogen target environments. Crop Science 37, 1110–1117.

Basford K.E., Cooper M., 1998. Genotype x environment interactions and some considerations for wheat breeding in Australia. Australian Journal of Agricultural Research 49, 153–174.

Berg T., 1997. Devolution of plant breeding. Http://www.idrc.ca/books/focus/833/berg.html.

Bertin P., Gallais A., 2000. Genetic variation for nitrogen use efficiency in a set of recombinant maize inbred lines I. Agrophysiological results. Maydica 45, 55–66.

Brancourt-Hulmel M., Heumez E., Pluchard P., Beghin D., Depatureaux C., Giraud A., Le Gouis J., 2005. Indirect vs. direct selection of winter wheat for low-input or high-input levels. Crop Science 45, 1427–1431.

Ceccarelli S., 1996a. Adaptation to low/high input cultivation. Euphytica 92, 203–214.

Ceccarelli S., 1996b. Positive interpretation of genotype by environment interactions in relation to sustainability and biodiversity. In: Cooper M., Hammer G.L. (eds.), Plant adaptation and crop improvement, CAB International. pp. 467–486.

Ceccarelli S., Grando S., 1999. Decentralized participatory plant breeding. Ileia newsletter December, 36–37.

Ceccarelli S., Grando S., Bailey E., Amri A., El-Felah M., Nassif F., Rezgui S., Yahyaoui A., 2001. Farmer participation in barley breeding in syria, morocco and tunisia. Euphytica 122(3), 521–536.

Ceccarelli S.S., 1994. Specific adaptation and breeding for marginal conditions. Euphytica 77, 205–219.

Cleveland D.A., Soleri D., Smith S.E., 1999. Farmer plant breeding from a biological perspective: Implications for collaborative plant breeding. CIMMYT. Economics working paper no 10.

Conroy C., Sutherland A., Martin A., 1999. Conducting farmer participatory research: What, when and how. In: Grant I.F., Sear C. (eds.), Decision Tools for Sustainable Development, Natural Resources Institute, Chatham, U.K. pp. 12–45.

Cooper M., Brennan P.S., Sheppard J.A., 1996. Positive interpretation of genotype by environment interactions in relation to sustainability and biodiversity. In: Cooper M., Hammer G.L. (eds.), Plant adaptation and crop improvement, CAB International. pp. 487–511.

Cooper M., Woodruff D.R., Eisemann R.L., Brennan P.S., DeLacy I.H., 1995. A selection strategy to accommodate genotype-by-environment interaction for grain yield of wheat: Managed-environments for selection among genotypes. Theoretical and Applied Genetics 90, 492–502.

Desclaux D., 2005. Participatory plant breeding methods for organic cereals. In: Lammerts Van Bueren E.T., Ostergard H. (eds.), Proceedings of the COST SUSVAR/ECO-PB Workshop on Organic Plant Breeding Strategies and the Use of Molecular Markers. Driebergen (NK), 17-19 January 2005, pp. 17–23.

Desclaux D., Hédont M. (Eds.), 2006. Proceedings of the ECO-PB Workshop on Participatory Plant Breeding: Relevance for Organic Agriculture?, Workshop held in Domaine de la Besse, France, 11-13 June 2006. European Consortium for Organic Plant Breeding, Institut Technique de l'Agriculture Biologique, Paris, France.

Fitzgerald D., 1993. Farmers deskilled: Hybrid corn and farmers' work. Technology and Culture 34(2), 324–343.

Gyawali S., Sunwar S., Subedi M., Tripathi M., Joshi K.D., Witcobe J.R., 2007. Collaborative breeding with farmers can be effective. Field Crops Research 101, 88–95.

Haugerud A., Collinson M.P., 1990. Plants, genes and people: Improving the relevance of plant breeding in Africa. Experimental Agriculture 26, 341–362.

Joshi A., Witcombe J.R., 1996. Farmer participatory crop improvement II. participatory varietal selection, a case study in India. Expl. Agric. 32, 461–477.

Morris M.L., Bellon M.R., 2004. Participatory plant breeding research: Opportunities and challenges for the international crop improvement system. Euphytica 136, 21–35.

Murphy K.M., Campbell K.G., Lyon S.R., Jones S.S., 2007. Evidence of varietal adaptation to organic farming systems. Field Crops Research 102, 172–177.

Murphy K.M., Lammer D., Lyon S.R., Carter B., Jones S.S., 2005. Breeding for organic and low-input farming systems: An evolutionary-participatory breeding method for inbred cereal grains. Renewable Agriculture and Food Systems 20(1), 48–55.

Presterl T., Seitz G., Landbeck M., M. T.E., W. S., H. G.H., 2003. Improving nitrogen-use efficiency in European maize: Estimation of quantitative genetic parameters. Crop Science 43(4), 1259–1265.

Riley K.W., 2003. Decentralized breeding and selection: Tool to link diversity and development. Http://www.idrc.ca/library/document/104582/riley.html.

Smith M., Weltzien E., 2000. Scaling-up in participatory plant breeding. In: Almekinders C., De Boef W. (eds.), Encouraging Diversity, Intermediate Technology Publications. pp. 208–213.

Smith M.E., Castillo G. F., Gomez F., 2001. Participatory plant breeding with maize in Mexico and Honduras. Euphytica 122(3), 551–565.

Sperling L., Ashby J.A., Smith M.E., Weltzien E., McGuire S., 2001. A framework for analyzing participatory plant breeding approaches and results. Euphytica 122, 439–450.

Sperling L., Loevinsohn M.E., Ntabomvura B., 1993. Rethinking the farmer's role in plant breeding: local bean experts and on-station selection in Rwanda. Experimental Agriculture 29, 509–519.

Sthapit B., Joshi K.D., Witcombe J.R., 1996. Farmer participatory crop improvement. III. participatory plant breeding, a case study for Nepal. Expl. Agric. 32, 479–496.

Sthapit B.R., Jarvis D., 1999. Participatory plant breeding for on-farm conservation. Ileia newsletter December, 40–41.

Suneson C.A., 1956. An evolutionary plant breeding method. Agronomy Journal 48, 188–191.

Ud-Din N., Carver B.F., Clutter A.C., 2004. Genetic analysis and selection for wheat yield in drought-stressed and irrigated environments. Euphytica 62(2), 89–96.

van de Fliert E., Braun A.R., 2002. Conceptualizing integrative, farmer participatory research for sustainable agriculture: From opportunities to impact. Agriculture and Human Values 19, 25–38.

van Ginkel M., Ortiz-Monasterio I., Trethowan R., Hernandez E., 2001. Methodology for selecting segregating populations for improved N-use efficiency in bread wheat. Euphytica 119(1), 223–230.

Wade L.J., McLaren C.G., Samson B..K., Regmi K.R., Sarkarung S., 1996. The importance of environmental characterization for understanding genotype by environment interactions. In: Cooper M., Hammer G.L. (eds.), Plant adaptation and crop improvement, CAB International. pp. 549–562.

Witcombe J.R., 1996. Participatory approaches to plant breeding and selection. Biotechnology and Development Monitor 29, 26–32.

Witcombe J.R., 1999. Do farmer participatory methods apply more to high potential areas than to marginal ones? Outlook on Agriculture 28(1), 43–49.

Witcombe J.R., Gyawali S., , Sunwar S., Sthapit B.R., Joshi K.D., 2005a. Participatory plant breeding is better described as highly client-oriented plant breeding. II. Optional farmer collaboration in the segregating generations. Expl. Agric. 42, 79–90.

Witcombe J.R., Joshi A., Joshi K.D., Sthapit B.R., 1996. Farmer participatory crop improvement. i. varietal selection and breeding methods and their impact on biodiversity. Expl. Agric. 32, 445–460.

Witcombe J.R., Joshi K.D., Gyawali S., Musa A.M., Johansen C., Virk D.S., Sthapit B.R., 2005b. Participatory plant breeding is better described as highly

client-oriented plant breeding. I. Four indicators of client-orientation in plant breeding. Expl. Agric. 41, 299–319.

Witcombe J.R., Virk D.S., 2001. Number of crosses and population size for participatory and classical plant breeding. Euphytica 122(3), 451–462.

Chapter 7

Assessing farmer interest in participatory plant breeding: Who wants to work with scientists?

Originally published as: Dawson J.C., Goldberger J., 2008b. Assessing farmer interest in participatory plant breeding: Who wants to work with scientists? Renewable Agriculture and Food Systems 23(1), 1-11. DOI 10.1017/S1742170507002141.

7.1 Abstract

Participatory research, particularly participatory plant breeding (PPB), can increase the relevance of public-sector research to the agricultural community. PPB has mostly been used in developing countries with resource-poor farmers, but there is increasing interest among farmers in developed countries who are dissatisfied with

the performance of available varieties. In 2006, scientists associated with the winter and spring wheat breeding programs in the Department of Crop and Soil Sciences and the Department of Community and Rural Sociology at Washington State University (WSU) conducted a survey of members of the Washington Association of Wheat Growers. Through analysis of the survey results, we sought to understand (1) whether or not farmers want to work with scientists in PPB programs and (2) the determinants of PPB interest. Results indicated that 52% of Washington wheat growers were interested in working with WSU scientists in a participatory breeding program. Interested farmers tended to be younger, college educated with fewer years of farming experience. Moreover, PPB interest appeared to be related positively to farm size, the number of wheat varieties planted, use of and interest in alternative production and marketing practices (e.g., seed saving, organic agriculture), and prior experience with WSU. Based on this analysis and ongoing discussions with farmers, we hope to develop a participatory wheat breeding program where farmers are able to choose their level of involvement with the breeding process based on their interest and needs. This new program will increase the relevance of WSUs wheat breeding programs to farmers in the state and could serve as a model for other public agricultural research programs seeking to increase farmer involvement and, thereby, democratize agricultural research.

Key words: democratization of science, participatory research, participatory plant breeding, wheat production

7.2 Introduction

The traditional mission of US Land-Grant universities dictates that scientists conduct research of direct relevance to people and communities. To accomplish this task, it is important for the public to take an active role in setting research priorities, participating in actual research projects and providing feedback about the consequences of research outputs. Some scholars and activists argue that citizen involvement can contribute to the democratization of university science and technology decision-making (Lacy and Glenna, 2006; Kleinman, 2000). When citizens are involved in the research process, research results are more likely to be relevant, accepted and put into action. Examples of citizen involvement include consensus conferences, science shops (i.e., entities that conduct independent, participatory research in response to concerns expressed by civil society), participatory action research and communitybased research (Lacy and Glenna, 2006). Other scholars have focused specifically on the need for more democratic participation in the public agricultural research enterprise (Middendorf and Busch, 1997; Busch and Lacy, 1983; Kloppenburg, 1991; Hassanein, 1999; Ostrom and Jackson-Smith, 2005). They argue that farmers, the primary beneficiaries of most research conducted in colleges of agriculture at Land-Grant universities, should be actively engaged in the research process. Participatory plant breeding (PPB), the focus of this paper, is one example of how scientists and farmers are attempting to democratize public agricultural research.

PPB uses both scientists and farmers knowledge to develop crop varieties suited to particular agro-ecological zones. In PPB projects, scientists and farmers work together to set breeding objectives, generate genetic variability, make selections, evaluate experimental varieties and generate and disseminate seeds. Agricultural scientists interested in these types of participatory research approaches certainly want farmers involved in their programs. However, less is known about why farmers, especially in more developed agricultural areas such as the United States and Europe, want to work with scientists in participatory research projects. What distinguishes those farmers who indeed want to work with university scientists? For example, are small-scale farmers, who are often the targeted beneficiaries of participatory breeding projects in developing countries, more interested than large-scale farmers? Are alternative farmers more interested than conventional farmers? Are farmers with pre-existing Land-Grant University ties more interested than farmers who have had little or no contact with public agricultural scientists and extension specialists? These and related questions form the basis of the analysis presented in this paper.

Specifically, we analyze data from a survey of Washington wheat growers to better understand (a) whether or not farmers want to work with scientists in participatory breeding programs and (b) the determinants of interest in PPB. We consider six sets of potential determinants of interest: farmer characteristics (e.g., gender, age, education), farm characteristics (e.g., farm size, wheat acreage, farm organization), production practices (e.g., number of wheat varieties planted, seed

saving, interest in certified organic production), marketing strategies (e.g., niche marketing, marketing clubs), experience working with university researchers and extension specialists, and opinions about university research and extension. Most PPB programs in developed countries have been initiated at the request of relatively small-scale organic farmers involved in organizations with ties to research universities. However, other types of farmers might also be interested in PPB. We hope this study will help broaden our understanding of whether and why farmers in developed countries are interested in working with university scientists in PPB programs.

7.3 From Formal to Participatory Plant Breeding

Formal plant breeding is conducted by professional scientists employed primarily by public agricultural research institutions or the private sector. These scientists set breeding goals, make crosses, test for desired traits, select superior lines and release new varieties without the organized participation of farmers. While most professional plant breeders make an effort to understand farmers needs, they rarely include farmers in the day-to-day decisionmaking of formal breeding programs. Formal plant breeding rests on the assumptions that farmers are not capable of making crosses and keeping track of progeny lines (Fitzgerald, 1993) and professional plant breeders are better equipped to use complex selection systems to select superior varieties (Witcombe et al., 2005). Plant breeders may also believe using farmers

management practices complicates experimental design and analysis (Haugerud and Collinson, 1990). Formal plant breeding became the norm in many countries because of increased scientific understanding of genetic principles, the industrialization of agriculture and investment in national agricultural research programs.

While it is true plant breeders have training in selection theory and experimental design, farmers also have valuable knowledge about environmental conditions and the characteristics that make a variety successful in their region. Many farmers already do their own research in testing and adapting new ideas and technologies (Conroy et al., 1999). Farmers in marginal agricultural environments often maintain and improve varieties developed over centuries of on-farm selection. Making use of both farmer and researcher knowledge can increase the relevance and efficiency of breeding programs. Therefore, over the past two decades, participatory approaches have gained popularity within formal international plant breeding programs.

PPB seeks to reverse the historical trend of separation between farmers and plant breeders, bringing them together in the process of developing new crop varieties or improving existing ones (Cleveland and Soleri, 2002). PPB arose out of the realization that many farmers in marginal production areas were not benefiting from conventional plant breeding programs. While modern varieties developed by conventional breeders have been widely adopted, they are grown primarily in areas of high agricultural potential. These varieties were selected in such high-potential

environments and are well adapted to these systems. As a result, the adoption of modern varieties has been very low in complex, diverse and risk-prone environments (Ceccarelli and Grando, 2007). PPB emphasizes collaboration and knowledge sharing between farmers and scientists as essential for identifying and improving suitable varieties for these marginal environments (Murphy et al., 2005). Although PPB usually refers to farmer participation in selecting from diverse plant populations, farmer participation can take other forms including helping to set research priorities and breeding goals, engaging in on-farm field trials and providing feedback on released varieties. Different farmers may wish to participate in different stages of the process depending on their interests and time availability.

Because PPB was originally developed for farmers on marginal land in developing countries (Duvick, 2004), some question if it is relevant to agricultural systems in favorable environments (Sperling et al., 2001; Witcombe, 1999). The farms associated with these systems tend to be large-scale, capital-intensive and oriented toward commodity markets. The use of high-yielding modern varieties is the norm, and little if any of the farm output is for the farmers own consumption. The use of farm inputs such as fertilizers and pesticides makes growing conditions similar from farm-to-farm and region-to-region, so a few varieties may perform well over a wide spectrum of environmental conditions. However, restricting genetic diversity to only a few varieties increases the vulnerability of agricultural systems to disease or pest epidemics and environmental stress. A single production practice or crop variety

will not be universally effective (Brummer, 2004).

While formal plant breeding programs have produced very successful varieties for developed agricultural systems, this does not mean these systems would not benefit from increased participation of farmers in the process of crop improvement. Highly productive areas have the potential for greater diversity in crop species and varietal diversity within species (Witcombe, 1999). This diversity can include both specific adaptation to the biological and physical environment, and suitability for specific markets and end-uses (Chiffoleau and Desclaux, 2006). Breeding crops adapted to specific farming systems and ecological zones is important for the sustainability of these systems and will require decentralized and participatory breeding programs to address the needs of a diverse landscape. Moreover, farmers have become increasingly interested in reducing the use of inputs (for both environmental and economic reasons) and finding alternatives to the conventional commodity system.

There are many examples of small-scale farmers in developing countries (Ceccarelli and Grando, 2007; Gyawali et al., 2007; Almekinders and Elings, 2001; Smith et al., 2001; Sthapit et al., 1996; Sperling et al., 1993) and alternative agriculturalists in developed agricultural systems (Sligh and Lauffer, 2004; Carena, 2005; Desclaux and Hédont, 2006) engaging in PPB programs. A case study of participatory wheat breeding in southern France, for example, found PPB was primarily of interest to organic farmers and had become a political strategy for

farmers associations that felt conventional breeding programs were not able to meet the needs of more sustainable agricultural systems. PPB was seen as a means for farmers to regain independence in their choice of varieties (Chiffoleau and Desclaux, 2006). Less represented in the literature are examples of PPB projects focused on larger-scale conventional farms in developed countries. Our study focuses on conventional Washington wheat growers, a majority of whom have been satisfied with university breeding programs. We hope this study will help broaden our understanding of why farmers in these systems might be interested in PPB approaches. Moreover, we offer the first analysis (to our knowledge) of some of the determinants of farmer interest in PPB in developed agricultural systems.

7.4 Wheat Production and Breeding in Washington State

According to the 2002 Census of Agriculture, Washington has 3,414 farms producing wheat for grain on 2,355,451 acres (953,217 ha) (US Department of AgricultureNational Agricultural Statistics Service (USDA – NASS), 2006). Most wheat growers are located in the eastern two-thirds of the state. The value of wheat production in the state was $456,316,000 in 2005 (US Department of AgricultureNational Agricultural Statistics Service (USDA – NASS), 2007). In terms of production value, wheat is the fifth most important agricultural commodity in the

state. Washington wheat growers produce 6.6% of all US wheat. Whitman County (the location of WSU) produces more wheat than any other county in the US (US Department of AgricultureNational Agricultural Statistics Service (USDA – NASS), 2007).

There is a high degree of differentiation and strict quality standards for each market class of wheat. Common market classes in eastern Washington are hard red (used for bread and Asian noodles) and soft white (used for pastries, crackers and other baked goods). Hard white wheat (used for whole wheat bread and noodle products) is a newer market segment beginning to attract growers interest. Based on roundtable discussions with wheat growers, it appears interest in alternative marketing strategies is driven by the expectation for a higher economic return from niche or specialty markets compared to the conventional commodity market.

Many farmers know environmental conditions influence quality. Moreover, they know matching varieties and market classes to particular environmental conditions can improve both quality and consistency. The lower rainfall zones (150400 mm per year) of eastern Washington produce high quality bread wheat, while the higher rainfall zones (up to 600 mm per year) produce excellent pastry wheat. These geographic advantages are lost if multiple wheat varieties from multiple locations within the state are mixed in the commodity system. Thus, some farmers are interested in identity-preserved marketing whereby specific varieties are grown for quality and sold at a premium.

Because of the wide range of environmental conditions in eastern Washington, scientists associated with the WSU winter wheat breeding program believe participatory methods could be appropriate for developing new varieties. While there are breeding nurseries and varietal evaluation trials throughout eastern Washington, the diversity of environments makes it very difficult for the program to develop varieties specifically for all farming systems and microclimates. Thus, in 2003, WSU scientists began working closely with a farmer in the dryland wheatfallow cropping system. They have since expanded their program to include three other farmers. Genetically diverse populations of wheat have been developed using an evolutionary participatory approach, which combines natural selection and site-specific farmer selection (Murphy et al., 2005).

In an effort to reach more interested farmers, scientists associated with WSUs winter and spring wheat breeding programs decided to conduct a mail survey of wheat growers in the state. The survey (discussed in more detail below) was designed to improve the relevance of the breeding program through a better understanding of farmer production practices, priorities and attitudes.

7.5 Methods

A mail survey of Washington wheat growers was conducted from January through March 2006. The survey was designed and sponsored by the winter and

spring wheat breeding programs in the Department of Crop and Soil Sciences and faculty in the Department of Community and Rural Sociology at WSU. The survey was conducted with the cooperation of the WSUs Social and Economic Sciences Research Center.

Survey questions were developed after eliciting farmer input. Questions dealt with many of the issues that surfaced during roundtable discussions with farmers in five eastern Washington counties. The surveys objective was to better understand how farmers make decisions about new technologies, production practices and marketing strategies. The survey included questions about experiences with WSU representatives, opinions about WSUs wheat breeding programs, desirable traits for new wheat varieties, wheat marketing strategies, perceived farming challenges and factors contributing to successful wheat farming. Other questions addressed genetically-modified (GM) wheat, organic farming, the development of perennial wheat and interest in breeding wheat varieties in collaboration with WSU breeders. The hope was to use the surveys findings to improve the relevance of WSUs wheat breeding programs.

The sampling frame for the study was the Washington Association of Wheat Growers (WAWG) membership list. The WAWG list is representative of commercial farmers who grow wheat as their primary crop. Small-scale and certified organic growers may be underrepresented in WAWG because of preferences for grower associations that better serve their information and networking needs. Nonetheless,

because scientists associated with the WSU wheat breeding program work closely with commercial wheat farmers in eastern Washington, the WAWG list was deemed an appropriate sampling frame for this particular study. Moreover, use of the WAWG list provided the opportunity to investigate PPB interest among a population of growers who are not typically the focus of PPB research.

With permission from the WAWG Board of Directors, questionnaires titled Wheat Production in Washington: Your Experiences with WSU and Input for Future Directions, cover letters, and business reply envelopes were mailed to all 1374 WAWG members on 14 February 2006. Survey procedures followed the Tailored Design Method (Dillman, 2000). Reminder postcards were sent out on 21 February 2006. Two weeks later (7 March 2006), second copies of the questionnaire were sent to non-respondents. Three hundred and seven (307) individuals were excluded because of ineligibility, bad addresses, and other reasons. The result was a corrected sample of 1067 growers. Of these, 553 wheat growers returned completed questionnaires. The completion rate for the survey was 51.8%, which is quite high for this type of farmer survey.

In this paper, our dependent variable is *interest in PPB*. It was measured by the following survey question: How interested are you in working directly with a WSU scientist in a participatory wheat breeding program within the next 13 years? A box appeared next to the question with the following information: Participatory wheat breeding uses both breeder and farmer expertise to develop varieties

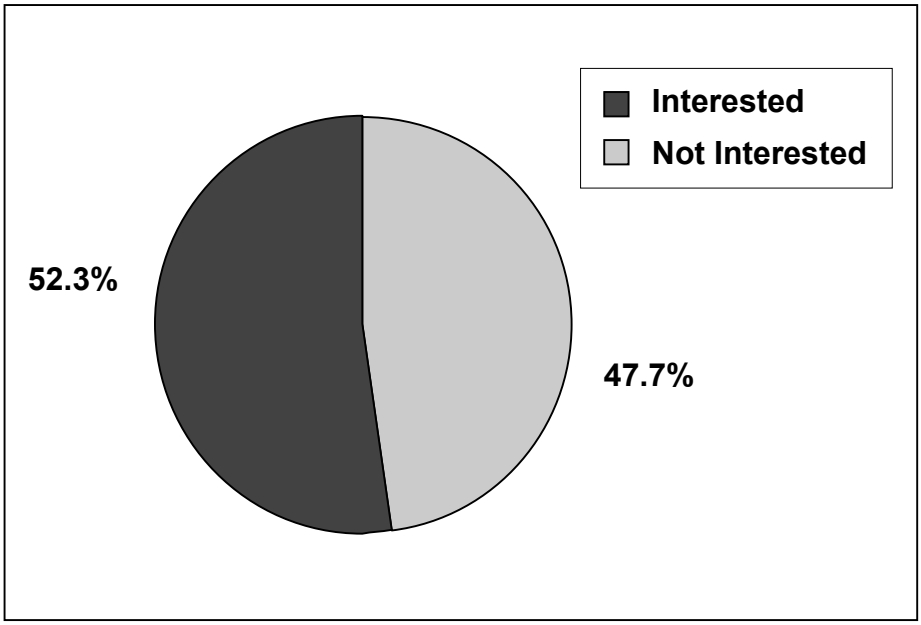


Figure 7.1: Interest in working with Washington State University (WSU) scientists in participatory wheat breeding programs, Washington wheat growers, 2006

particularly suited to a specific set of environmental challenges. Answer categories included very interested, somewhat interested somewhat uninterested, and very uninterested. We created a dichotomous variable by grouping the first two categories (into interested) and the last two categories (into not interested). Four hundred and ninety three (493) respondents provided valid answers to the question. Results indicate that 258 growers (52.3% of respondents) are interested in working with a WSU scientist in a participatory wheat-breeding program, while 235 growers (47.7%) are not interested (see Fig. 7.1).

The primary objective of the analysis presented below is to explore the relationship between our dependent variable (interest in PPB) and six sets of

independent variables. Our independent variable groups include farmer characteristics, farm characteristics, production variables, wheat marketing variables, variables measuring experience with WSU researchers and extension specialists and variables measuring opinions about WSU research and extension. All of the independent variables presented in the tables were measured by direct survey questions. The continuous variables (such as age, years in farming, total acres farmed, etc.) have been recoded as categorical variables for the purposes of analysis. We conducted Pearson chi-square tests to determine whether growers interested in PPB and growers not interested in PPB differ significantly in various characteristics. The Pearson chi-square test is designed to test for independence between two nominal variables. The null hypothesis is that the two variables are statistically independent. The test is based on a comparison between the observed and expected frequencies in the cells of a cross-classification table.

7.6 Findings

Table 7.6 presents percentage distributions for farmers interest in PPB by four farmer characteristics: gender, age, education and years in farming. There are statistically significant differences in PPB interest for three of the four demographic variables. Younger farmers, as well as individuals with less farming experience, are more interested in PPB. Note, however, that these are not mutually exclusive groups

because of the high correlation (r = 0.81) between age and number of years in farming.

The data in Table 7.6 indicate a negative (and nearly linear) relationship between PPB interest and both age and number of years in farming. This could be due to a number of factors. Younger and newer farmers may be more willing to start a long-term project with the prospect of significant benefits down the road. For example, they may be looking for alternatives to conventional commodity production out of concern for the long-term economic and environmental viability of their operations. Younger and newer farmers may also be experimenting with different production practices and varieties as they get to know the specific conditions on their farms. Older and more experienced farmers, in contrast, may already know the types of varieties and production methods that work well on their farms and, consequently, may not be as interested in making significant changes to their operations.

The data in Table 7.6 also suggest that interest in PPB varies significantly by farmers level of education. Respondents with high school degrees (or less) are the least interested in PPB, while respondents with vocational degrees, college degrees and some postgraduate education exhibit the greatest PPB interest. One possible explanation for these findings is that more educated farmers are more aware of WSU research or plant breeding in general. Many farmers in Washington are WSU graduates, which could influence their interest in working with WSU scientists. Interestingly, only 43% of respondents with postgraduate degrees are interested in

Table 7.1: Percentage distribution of interest in PPB by farmer characteristics, Washington wheat growers, 2006

Farmer characteristics	N	% of total sample	Interested in PPB %	Chi-square
Gender				
Male	494	96.3	53.5	
Female	19	3.7	25.0	3.803
Age				
Under 45	58	11.4	70.4	
45 – 54	173	34.1	59.0	
55 – 64	148	29.1	54.5	
65 and over	129	25.4	29.9	31.738***
Education				
High school degree or less	50	9.8	28.6	
Some college	131	25.8	38.9	
Vocational degree	51	10.0	57.8	
College degree	206	40.6	64.7	
Some postgraduate work	30	5.9	59.3	
Postgraduate degree	40	7.9	43.2	31.621***
Number of years in farming				
Less than 15	39	7.7	77.8	
15 – 29	179	35.4	60.7	
30 – 44	193	38.1	50.9	
45 or more	95	18.8	30.1	30.513***
*P<0.05; **P<0.01; ***P<0.001 (Pearson chi-square test)				
1The average age of survey respondents was 57.5 years 2 The average number of years in farming was 32.0 years				

PPB.

Table 7.6 presents percentage distributions for farmers interest in PPB by farm size, winter and spring wheat acreage, farm business organization, total farm receipts and percentage of household income from farming. Chi-square results indicate a statistically significant relationship between PPB interest and farm size, but not farm business organization or the percentage of household income from farming. The

most intriguing finding is that farmers interest in PPB increases with farm size, regardless of whether measured in terms of total acres farmed, wheat acres, or farm receipts. This finding does not support the common perception that smaller growers are the ones most interested in participatory research (Sligh and Lauffer, 2004; Carena, 2005; Desclaux and Hédont, 2006). There are several possible explanations for our finding that larger-scale farmers seem to be more interested in PPB. First, farmers with more acreage may be more likely to have some marginal land, for which they want to work with scientists to develop special varieties. Secondly, large-scale farmers (compared to small-scale farmers) may have access to more resources and hired help, resulting in their ability to devote more time to participatory breeding projects. Thirdly, it is possible that some larger-scale farmers were able to expand their operations because of successful experimentation with new practices. These farmers may look favorably upon opportunities to experiment further.

Another interesting finding from Table 7.6 is the lack of a statistically significant relationship between PPB interest and the percentage of household income from farming. Farmers who rely on farm receipts for their entire income and those with off-farm income sources appear to be equally interested in participatory breeding programs. Off-farm income might provide a financial cushion to allow farmers to assume the risks of a participatory breeding program. However, farmers with off-farm jobs may face time constraints that could negatively affect their ability to participate in plant breeding activities. In contrast, farmers who rely solely on

farming for their household income might have the incentive and flexibility (especially in terms of labor time) to develop varieties for specific environmental conditions on their farms.

Table 7.6 presents percentage distributions for farmers interest in PPB by various production variables. We find statistically significant chi-square results for all but two of the variables included in the table. Respondents who planted three or more public wheat varieties were more likely to be interested in PPB. Perhaps these growers are more aware of differences in variety performance across different sections of their farms. Growers who agree that specific wheat varieties should be grown only in appropriate geographic locations due to quality concerns are more likely to exhibit PPB interest. This lends additional support to the hypothesis that farmers with PPB interest are more aware of the environmental effects on varietal performance and the need for wheat varieties adapted to specific agro-ecological zones.

The data in Table 7.6 also suggest farmers who are interested in alternative production methods are more inclined to want to breed new varieties in partnership with scientists. First, farmers who save seed from wheat and other crops are more likely than farmers who do not engage in seed saving to be interested in PPB. Farmers save seed for many reasons, including the desire to be self-sufficient or to avoid the cost of purchasing seed each year. Since farmers would have control of the varieties developed through PPB, it is not surprising that farmers who want to control their own seed supply seem to be very interested in PPB. Secondly, there is a

Table 7.2: Percentage distribution of interest in PPB by farm characteristics, Washington wheat growers, 2006

Farm characteristics	N	% of total sample	Interested in PPB (%)	Chi-square
Total acres farmed				
1 – 1000 acres	77	15.0	29.0	
1001 – 2000 acres	133	25.9	48.7	
2001 – 3000 acres	115	22.4	53.8	
3001 – 4000 acres	80	15.6	54.7	
4001 – 5000 acres	36	7.0	73.3	
5001 acres or more	73	14.2	63.8	24.777***
Acres of winter wheat in 2005				
0 – 500 acres	139	27.2	37.2	
501 – 1000 acres	143	28.0	56.4	
1001 – 1500 acres	102	20.0	56.2	
1501 or more	127	24.9	60.7	15.800***
Acres of spring wheat in 2005				
None	217	43.7	45.5	
1 – 250 acres	123	24.7	59.1	
251 – 500 acres	70	14.1	48.4	
501 or more	87	17.5	65.8	11.894**
Farm business organization				
Single family or individual operation	147	29.1	43.8	
Family partnership	112	22.1	54.1	
Family corporation	221	43.7	58.2	
Other	26	5.1	47.8	6.829
Total farm receipts in 2005 ($)				
Less than 25,000	22	4.6	36.8	
25,000 – 49,999	19	4.0	29.4	
50,000 – 99,999	37	7.7	37.5	
100,000 – 249,999	172	35.9	50.0	
250,000 – 499,999	146	30.5	59.8	
500,000 or more	83	17.3	59.5	13.070*
% of household income from farming				
024%	72	14.4	51.6	
2549%	67	13.4	56.4	
5074%	98	19.6	55.6	
75100%	264	52.7	50.8	0.956
*P<0.05; **P<0.01; ***P<0.001 (Pearson chi-square test)				
[1] The average total acreage was 3145 acres [2] The average winter wheat acreage was 1183 acres [3] The average spring wheat acreage was 280 acres.				

statistically significant relationship between PPB interest and interest in transitioning to certified organic production. This is not altogether surprising because the growers who originally worked with the WSU winter wheat breeding program were organic producers. These growers may be turning to organic production as a means to reduce their dependence on external inputs (including seeds) or to sell high quality wheat in alternative markets. Moreover, some growers may be interested in PPB as a result of their perception that varieties developed on-farm would perform better than existing varieties in organic systems. In fact, this perception has motivated organic farmers in Europe and the US to initiate PPB activities.

The data in Table 7.6 also suggest interest in planting GM wheat varieties is not related to interest in participatory breeding. Wheat growers may consider both participatory breeding and the development of GM varieties as strategies for improving plant varieties. This interpretation contradicts findings from case studies of participatory breeding in the US and Europe where growers were interested in participatory breeding as a method of ensuring control over seed supplies and preventing GM contamination of seed stocks (Chiffoleau and Desclaux, 2006; Sligh and Lauffer, 2004; Carena, 2005; Desclaux and Hédont, 2006).

Table 7.6 presents percentage distributions for farmers interest in PPB by three wheat-marketing variables. Specifically, respondents were asked to indicate their level of interest in three wheat-marketing strategies: maintaining the current commodity

Table 7.3: Percentage distribution of interest in PPB by production variables, Washington wheat growers, 2006

Production variables	N	% of total sample	Interested in PPB (%)	Chi-square
Private wheat varieties planted in 2005				
None	274	55.4	49.0	
1	132	26.7	60.0	
2	61	12.3	52.7	
3 or more	28	5.7	72.0	7.610
Public wheat varieties planted in 2005				
None	27	5.5	54.5	
1	119	24.3	42.2	
2	173	35.3	50.0	
3 or more	171	34.9	66.2	16.450***
Typically plant wheat seed saved from own fields				
No	316	61.5	47.6	
Yes	198	38.5	62.1	9.352**
Typically save seed for other crops (besides wheat)				
No	404	80.3	49.7	
Yes	99	19.7	65.9	7.474**
Interest in transitioning to certified organic production				
Not interested	457	86.1	49.3	
Interested	74	13.9	75.8	16.011***
Interest in planting GM wheat varieties				
Not interested	170	34.2	48.0	
Interested	327	65.8	53.8	1.359
Agreement with statement: Specific wheat varieties should be grown only in appropriate geographic areas due to quality concerns'				
Strongly disagree	12	2.3	44.4	
Somewhat disagree	70	13.1	43.1	
Somewhat agree	308	57.8	50.4	
Strongly agree	143	26.8	66.4	12.998**
*P<0.05; **P<0.01; ***P<0.001 (Pearson chi-square test)				

system, niche marketing of high value wheat varieties and establishing marketing clubs to pool varieties for sale to end users. We find no statistically significant difference in PPB interest for farmers with different levels of interest in maintaining the current commodity system. In fact, 65% of the respondents with no interest in maintaining the commodity system and nearly 64% of those with extreme interest in maintaining the system are interested in participating in breeding programs. It is likely farmers who are interested in keeping the current commodity system, but also interested in participating in breeding programs, are concerned with reducing input costs. These farmers may see the development of their own wheat varieties as a way of reducing costs through varietal adaptation to specific environmental conditions. For example, if a farmer chooses to conduct selection with reduced herbicides or pesticides, the variety developed will most likely have improved tolerance to weed pressure and resistance to diseases or insects. Thus, over time, selection for low-input systems can lower input costs for the same yield and quality goals.

The data in Table 7.6 also indicate a positive (and somewhat linear) relationship between PPB interest and interest in the two alternative marketing strategies: niche marketing and marketing clubs. There are several reasons why growers interested in alternative marketing strategies might be more interested in PPB. Growers may want to develop a specialty product that could be niche-marketed. Moreover, they may be interested in improving grain quality for direct marketing to end-users who value nutritional value and food product quality

Table 7.4: Percentage distribution of interest in PPB by wheat marketing variables, Washington wheat growers, 2006.

Wheat marketing variables	N	% of total sample	Interested in PPB %	Chi-square
Interest in maintaining current commodity system				
Not interested	24	4.3	65.0	
Slightly interested	143	25.9	53.8	
Somewhat interested	269	48.6	49.8	
Extremely interested	84	15.2	63.5	5.397
Interest in niche marketing of high-value wheat varieties or products				
Not interested	22	4.0	35.0	
Slightly interested	120	21.7	43.9	
Somewhat interested	209	37.8	48.4	
Extremely interested	171	30.9	69.7	24.958***
Interest in marketing club that pools specific varieties to sell directly to end users				
Not interested	31	5.6	28.6	
Slightly interested	147	26.6	46.3	
Somewhat interested	235	42.5	52.2	
Extremely interested	113	20.4	74.5	28.050***
*P<0.05; **P<0.01; ***P<0.001 (Pearson chi-square test)				

over yield and protein content. They may also see PPB as a means to diversify and lower input costs.

Table 7.6 presents percentage distributions for farmers interest in PPB by several variables measuring farmers experiences with WSU researchers/extension specialists and their programs. We find statistically significant chisquare results for all six variables included in the table. Not surprisingly, respondents who reported having had the most contact with WSU researchers and extension specialists expressed the greatest interest in working with WSU scientists in participatory breeding programs. Similarly, respondents who had attended one or more WSU field days were more interested in PPB compared to respondents who had not attended

any field days. PPB interest also appears to be related positively to the degree of importance attributed to WSU extension specialists, researchers and field days as sources of information for decisions about growing wheat.

The data in Table 7.6 also indicate PPB interest is greater among farmers familiar with the WSU effort to breed perennial wheat compared to farmers unfamiliar with this effort. The objective of the perennial wheat-breeding project is to develop wheat plants that produce grain for multiple years. Although perennial wheat is still in the experimental stages, preliminary results have been presented at many wheat grower meetings. It is likely growers with an interest in the latest activities of the WSU wheat breeding programs (i.e., growers who attend grower meetings and other gatherings focused on WSU research programs) tend to express greater interest in new participatory breeding efforts.

Table 7.6 presents percentage distributions for farmers interest in PPB by several variables measuring farmers opinions about WSU research and extension programs. PPB interest does not appear to be related to the perceived degree to which WSU researchers and extension specialists have been successful at serving the needs of wheat growers. However, there is a positive relationship between PPB interest and the degree to which growers perceived that WSU research not adequately focused on farmer needs negatively affected their farm operations. In other words, growers who reported being highly affected by a lack of relevant WSU research were the most likely to be interested in working with scientists to develop

Table 7.5: Percentage distribution of interest in PPB by variables measuring experience with WSU, Washington wheat growers, 2006

Experience with WSU	N	% of total sample	Interested in PPB (%)	Chi-square
Contact with WSU researchers				
Not at all	212	40.5	37.2	
Once a year or less	175	33.4	53.8	
More than once a year	137	26.1	75.4	48.853***
Contact with WSU extension specialists				
Not at all	147	27.3	33.6	
Once a year or less	167	31.0	53.0	
More than once a year	224	41.6	65.2	31.755***
Importance attributed to WSU extension agents/scientists as source of information for decisions about growing wheat				
Not important	109	21.8	31.0	
Slightly important	215	43.0	45.4	
Mostly important	142	28.4	52.8	
Extremely important	34	6.8	70.0	20.251***
WSU field days attended (20012005)				
None	149	28.7	34.4	
12	123	23.7	43.1	
34	112	21.5	62.6	
5 or more	136	27.2	70.8	42.615***
Importance attributed to WSU field days as source of information for decisions about growing wheat				
Not important	169	33.5	33.3	
Slightly important	200	39.6	42.1	
Mostly important	103	20.4	52.7	
Extremely important	33	6.5	65.4	18.067***
Familiarity with WSU effort to breed perennial wheat				
Not familiar	252	52.8	44.9	
Familiar	282	47.2	60.3	11.613***
*P<0.05; **P<0.01; ***P<0.001 (Pearson chi-square test)				

new wheat varieties. This finding supports one of the key goals of PPB; to reach farmers who have not benefited from formal plant breeding programs. However, because the survey question pertained to WSU research (in general) rather than the development of wheat varieties (in particular), we must exercise caution in interpretation. Interestingly, we do not find statistically significant relationships between growers PPB interest and their level of satisfaction with WSUs winter and spring wheat breeding programs. Growers who feel negatively affected by lack of attention by WSU researchers could certainly benefit from participation in research specifically tailored to meet their needs for certain varietal characteristics. These growers may also be interested in participating in priority setting or discussions about the overall goals of breeding and research programs.

7.7 Summary and Conclusion

The objective of our study was to broaden our understanding of whether and why farmers in developed countries are interested in working with university scientists in participatory breeding programs. Based on our analysis of data from a survey of wheat growers in Washington, we found approximately 52% of growers were interested in participating in university breeding programs. This finding suggests that it is not just social scientists, activists and (some) scientists who want to democratize university science and technology decision-making. A majority of

Table 7.6: Percentage distribution of interest in PPB by variables measuring attitudes about WSU, Washington wheat growers, 2006

Attitudes about WSU	N	% of total sample	Interested in PPB (%)	Chi-square
Perceived degree to which WSU researchers have been successful at serving the needs of wheat growers				
Very unsuccessful	27	6.2	56.0	
Somewhat unsuccessful	48	11.0	57.5	
Somewhat successful	227	51.9	55.6	
Very successful	135	30.9	62.1	1.401
Perceived degree to which WSU extension specialists have been successful at serving the needs of wheat growers				
Very unsuccessful	14	3.2	61.5	
Somewhat unsuccessful	52	11.8	50.0	
Somewhat successful	227	51.6	55.8	
Very successful	147	33.4	59.7	1.493
Level of satisfaction with WSUs winter wheat breeding program				
Very dissatisfied	23	4.2	61.9	
Somewhat dissatisfied	77	13.9	54.5	
Somewhat satisfied	276	49.9	52.4	
Very satisfied	145	26.2	54.1	0.772
Level of satisfaction with WSUs spring wheat breeding program				
Very dissatisfied	15	2.7	53.8	
Somewhat dissatisfied	74	13.4	53.2	
Somewhat satisfied	277	50.1	51.6	
Very satisfied	144	26.0	58.2	1.553
Perceived degree to which WSU research not adequately focused on farmer needs negatively affected farm operation				
Not affected	95	18.2	46.0	
Hardly affected	183	35.1	47.9	
Somewhat affected	197	37.7	56.9	
Highly affected	47	9.0	73.8	11.820**
*P<0.05; **P<0.01; ***P<0.001 (Pearson chi-square test)				

farmersat least in Washington Statealso want a more participatory public agricultural research system. They themselves want to be actively engaged in the research process.

Many factors appear to be associated with growers desire to work with professional breeders. Younger, college educated farmers with fewer years of farming experience were more interested in PPB compared to farmers with more years of farming experience and either no advanced degree or a post-graduate degree. Our data also indicated PPB interest was related positively to farm size (whether measured in terms of total acres farmed, total wheat acres, or total farm receipts) and the number of wheat varieties planted. Growers use of and interest in alternative production and marketing practices (e.g., seed saving, organic farming, niche marketing and marketing clubs) were also related significantly to interest in participatory breeding. We found statistically significant chi-square results for all of our measures of growers prior experience with WSU (e.g., contact with researchers and extension specialists, number of field days attended, familiarity with WSUs effort to breed perennial wheat). Finally, our data indicated growers who reported being highly affected by inadequate attention from WSU researchers were the most likely to be interested in working with scientists to develop new wheat varieties.

Until we conduct further roundtable discussions and interviews with growers, we can only offer preliminary interpretations of our findings and generalizations about which farmers are most likely to be interested in PPB. It is essential to keep in

mind that farmers are an extremely diverse group. Different farmers will have different reasons for wanting to work with university plant breeders. Moreover, most farmers will pursue multiple strategies to ensure the success of their operation. PPB is likely to be one project among many contributing to the farm operation. We believe PPB is sufficiently adaptable to allow farmers to use it to achieve multiple goals. We hope PPB in Washington State and elsewhere will help farmers gain greater control of the development of varieties to meet their specific needs and desires. Increased farmer involvement in plant breeding has the potential to contribute to the democratization of research and technology development at public agricultural research institutions.

Acknowledgments

Funding for this research was provided by the Department of Crop and Soil Sciences (WSU), the Department of Community and Rural Sociology (WSU), the Washington Wheat Commission and graduate research fellowships provided by the National Science Foundation and the Land Institute. The authors thank the WAWG staff and board, as well as the farmers who participated in the mail survey and roundtable discussions. Invaluable survey assistance was provided by Rose Krebill-Prather at Washington State Universitys Social and Economic Sciences Research Center (SESRC). The authors also thank Stephen Jones, Ray Jussaume, Leland Glenna and two anonymous reviewers for their input and comments on this

manuscript. All opinions, findings, conclusions and recommendations expressed in this paper are those of the authors and do not necessarily reflect the views of the funding agents and individuals listed above.

Bibliography

Almekinders C.J.M., Elings A., 2001. Collaboration of farmers and breeders: Participatory crop improvement in perspective. Euphytica 122, 425–438.

Brummer E.C., 2004. Breeding for sustainable cropping systems. In: Lauffer L., Sligh M. (eds.), Summit on Seeds and Breeds for 21st Century Agriculture. Rural Advancement Foundation International-USA, pp. 63–70.

Busch L., Lacy W.B., 1983. Science, Agriculture and the Politics of Research. Westview Press, Boulder, CO.

Carena M.J. (Ed.), 2005. Abstracts of Panel Discussion on Developing Farmer-Breeder Teams. Session 310 of the American Society of Agronomy Annual Meeting, Salt Lake City, UT, 10 November 2005, Northern Plains Sustainable Agriculture Society and the Crop Science Society of America.

Ceccarelli S., Grando S., 2007. Decentralized participatory plant breeding: an example of demand driven research. Euphytica 155, 349–360.

Chiffoleau Y., Desclaux D., 2006. Participatory plant breeding: the best way to

breed for sustainable agriculture? International Journal of Agricultural Sustainability 4(2), 199–130.

Cleveland D.A., Soleri D., 2002. Farmers, scientists and plant breeding: knowledge, practice and the possibilities for collaboration. In: Cleveland D.A., Soleri D. (eds.), Farmers, Scientists and Plant Breeding: Integrating Knowledge and Practice, CAB International, Okon, UK. p. 118.

Conroy C., Sutherland A., Martin A., 1999. Conducting farmer participatory research: What, when and how. In: Grant I.F., Sear C. (eds.), Decision Tools for Sustainable Development, Natural Resources Institute, Chatham, U.K. pp. 12–45.

Desclaux D., Hédont M. (Eds.), 2006. Proceedings of the ECO-PB Workshop on Participatory Plant Breeding: Relevance for Organic Agriculture?, Workshop held in Domaine de la Besse, France, 11-13 June 2006. European Consortium for Organic Plant Breeding, Institut Technique de l'Agriculture Biologique, Paris, France.

Dillman D., 2000. Mail and Internet Surveys: The Tailored Design Method. John Wiley and Sons, New York, NY.

Duvick D., 2004. The current state of plant breeding: how did we get here? In: Sligh M., Lauffer L. (eds.), Summit proceedings: Summit on seeds and breeds for the 21st century. Rural Advancement Foundation International, USA, Pittsboro S.C.

Fitzgerald D., 1993. Farmers deskilled: Hybrid corn and farmers' work. Technology

and Culture 34(2), 324–343.

Gyawali S., Sunwar S., Subedi M., Tripathi M., Joshi K.D., Witcobe J.R., 2007. Collaborative breeding with farmers can be effective. Field Crops Research 101, 88–95.

Hassanein N., 1999. Changing the Way America Farms. University of Nebraska Press, Lincoln, NE.

Haugerud A., Collinson M.P., 1990. Plants, genes and people: Improving the relevance of plant breeding in Africa. Experimental Agriculture 26, 341–362.

Kleinman D.L. (Ed.), 2000. Science, Technology, and Democracy. State University Press of New York, Albany, NY.

Kloppenburg J.R., 1991. Social theory and the de/reconstruction of agricultural science: local knowledge for an alternative agriculture. Rural Sociology 56(4), 519–548.

Lacy W., Glenna L., 2006. Democratizing science in an era of expert and private knowledge. International Journal of Technology, Knowledge and Society 1(3), 37–45.

Middendorf G., Busch L., 1997. Inquiry for the public good: democratic participation in agricultural research. Agriculture and Human Values 14, 45–57.

Murphy K.M., Lammer D., Lyon S.R., Carter B., Jones S.S., 2005. Breeding for

organic and low-input farming systems: An evolutionary-participatory breeding method for inbred cereal grains. Renewable Agriculture and Food Systems 20(1), 48–55.

Ostrom M., Jackson-Smith D., 2005. Defining a purpose: diverse farm constituencies and publicly funded agricultural research and extension. Journal of Sustainable Agriculture 27(3), 5776.

Sligh M., Lauffer L. (Eds.), 2004. Summit proceedings: Summit on Seeds and Breeds for 21st century agriculture. Rural Advancement Foundation International, USA, Pittsboro S.C.

Smith M.E., Castillo G. F., Gomez F., 2001. Participatory plant breeding with maize in Mexico and Honduras. Euphytica 122(3), 551–565.

Sperling L., Ashby J.A., Smith M.E., Weltzien E., McGuire S., 2001. A framework for analyzing participatory plant breeding approaches and results. Euphytica 122, 439–450.

Sperling L., Loevinsohn M.E., Ntabomvura B., 1993. Rethinking the farmer's role in plant breeding: local bean experts and on-station selection in Rwanda. Experimental Agriculture 29, 509–519.

Sthapit B., Joshi K.D., Witcombe J.R., 1996. Farmer participatory crop improvement. III. participatory plant breeding, a case study for Nepal. Expl. Agric. 32, 479–496.

US Department of AgricultureNational Agricultural Statistics Service (USDA – NASS), 2006. 2002 census of agriculture. Available at: http://www.nass.usda.gov/Census_of_Agriculture/ (verified 6 October 2007).

US Department of AgricultureNational Agricultural Statistics Service (USDA – NASS), 2007. The pride of washington state. Pamphlet. Available at: http://www.nass.usda.gov/Statistics_by_State/Washington/Publications/wabro.pdf (verified 6 October 2007).

Witcombe J.R., 1999. Do farmer participatory methods apply more to high potential areas than to marginal ones? Outlook on Agriculture 28(1), 43–49.

Witcombe J.R., Joshi K.D., Gyawali S., Musa A.M., Johansen C., Virk D.S., Sthapit B.R., 2005. Participatory plant breeding is better described as highly client-oriented plant breeding. I. Four indicators of client-orientation in plant breeding. Expl. Agric. 41, 299–319.

Chapter 8

Participatory Research

8.1 Grower Roundtables and Survey

The breeding programs at WSU try to produce varieties that are well suited for all the wheat growing areas of the state, and tries to anticipate changes in pathogen races or market demands that will require new wheat varieties. One way to ensure that our breeding efforts are relevant to what farmers in Washington State need in the future is to ask growers what they would like to see as priorities, and what challenges they expect to face over the next several years.

A series of grower roundtables were held in counties throughout Eastern Washington. The purpose of these discussions was to interact directly with growers and to get their responses to the open-ended questions: What will farming in Eastern Washington look like in 10-20 years? What do you want it to look like? How

can our research help you get there? Farmers were recruited to participate through one of our collaborating growers in the county. It was not a random sample, but rather a selection of people who are interested in thinking about the larger questions facing agriculture in this region and willing to participate in a dialog with researchers. Preliminary conversations suggested that most farmers are pessimistic about the future of rural communities and family farming, but have hope that their farms can survive by changing the way they deal with marketing and production. Roundtables were held November 11, 2005 and November 30, 2006 in Whitman county: March 4, 2006 and November 30, 2006 in Franklin County; November 18, 2006; in Adams county, May 31, 2006 in Spokane County; and June 1, 2006 in Benton County. In addition, many farmers presented testimony at a listening session held by Secretary of Agriculture Mike Johanns on November 3, 2005, in Cheney, WA, leading up to the 2007 farm bill, and their comments are included in the discussion.

In addition to the roundtables, a mail survey of farmers in Eastern Washington was conducted. In the fall of 2005, the survey questions were developed with the help of several farmers. Questions dealing with many of the issues that surfaced in the roundtables were used to correlate the results of the survey with the smaller group discussions, as there were several opportunities to write in comments on the survey form in addition to multiple choice and ranking questions. The survey results can help us understand how farmers evaluate new technologies and practices in their production systems, including desirable traits for new wheat varieties, marketing

strategies, genetically modified wheat, organic farming, and perennial wheat. The survey also explored differences in management styles; whether farmers are most interested in maximizing production, minimizing inputs, capturing quality premiums, and/or conserving soil and other natural resources.

In collaboration with Rose Krebill-Prather at the Social and Economic Sciences Research Center (SESRC) at WSU, the survey questions were edited and formatted to form an 8 page booklet that took about 15-20 minutes for farmers to complete. Several farmers were asked to test the survey for clarity and ease of completion. The SESRC handled all mailings and data collection. Surveys were mailed to all members of the Washington Association of Wheat Growers, using the WAWG mailing list with permission from their Board of Directors. A cover letter and the survey were sent to each member on February 14, 2006, with a reminder postcard sent out on February 21 and a final mailing to non-respondents on March 7. Receipt of completed surveys for data collection was closed off on April 7th, 2006. There were a total of 1374 names on the list, of which 557 returned completed questionnaires, for a response rate of 41%. An additional 239 were returned as not eligible, giving a return rate of 61% and a completion rate of 52%, which is very high for a survey of this type.

8.1.1 Survey results

Over 80% of growers are currently satisfied with the winter and spring wheat breeding programs at WSU. Many of the results were not surprising, perhaps because we developed the questions with farmer input and advice. Ensuring high yields and lowering input costs were the two factors identified as most important for successful farming operations. The third most important factor was increasing the number of buyers and markets for wheat, which relates to the breeding program priority regarding wheat for specialized market segments. Limited market opportunities, too few companies buying wheat and low commodity prices were all ranked high on the list of negative challenges faced by wheat growers. A majority of growers were interested in alternative marketing strategies to overcome these challenges. These include marketing clubs to sell specific varieties directly to end users, niche marketing of high-value wheat varieties or products and rebuilding the regional processing and distribution infrastructure to allow more local control of the marketing process. Growers are very interested in potentially receiving a premium for delivering high quality clean wheat to buyers rather than going through standard distribution channels that may blend wheat of different qualities so that the blend just makes the grade.

In addition to covering the conventional breeding programs, the survey also included questions on our perennial and participatory projects. About half the growers were interested in participating in the wheat breeding program.

Participatory breeding methods have been successful in many areas of the world, and an evolutionary participatory breeding strategy may have the potential to make significant gains in breeding wheat for difficult environments or alternative production systems in Eastern Washington (Murphy et al., 2005). This question was the topic of an analysis to be covered in a later section. Perennial wheat is one of the long-term breeding objectives in the winter wheat program. It is less of a priority to growers than the annual breeding program, however, many growers wrote in comments supporting the development of perennial wheat.

8.2 Discussion of roundtable, listening session and survey comments

8.2.1 Future of farming and rural communities

There was significant concern about the future of wheat farming in Washington, especially related to the ability of young farmers to make a living through full time farming. Growers wanted their children to be able to farm, but did not feel that they should encourage an interest in farming with the current economic situation. Several said that if the younger generation wanted to farm, the older generation would have to quit because there was no way to support two households on one farm, and often the older generation did not have the resources to retire early

or simply was not ready to give up farming. Off-farm jobs are increasingly necessary to support farm families through added income and health insurance, but jobs are limited in rural communities, meaning one member of the family often has a long commute to work. In other cases, people live in more urban areas and the farmer commutes back to their farm or to rented land. While people enjoy living in rural areas, it is difficult to attract younger families, teachers and others to work in rural communities if there are not good schools and activities for children. A growing number of people want to live in rural areas while working elsewhere, or to retire there, so some of the best agricultural land close to urban areas is being taken up by "rural developments" while truly rural areas are losing population and businesses. Agricultural businesses in rural communities are not the only ones consolidating either, as many small businesses are being out-competed by national or international retailers, banks, pharmacies and shippers. Growers felt strongly about being able to continue to live in rural areas, citing intangible benefits such as birds singing in the morning, dark night skies with thousands of stars and the absolute quiet that is possible only away from large population centers.

The economics of farming are increasingly difficult from the perspective of farmers. In 2005, when most of the roundtables were conducted, the price of a bushel of wheat was lower than that of a gallon of gas. This situation improved in 2007, but cyclical cycles and erratic prices have always been part of commodity farming, and with each downward cycle, more farmers go out of business. In 2005, about 60

farmers in Eastern Washington went out of business, compared to an average of twelve farmers quitting each year and three or four new farmers starting. Farmers able to weather the bad times usually own their land and can eat into their equity, while farmers who must rent a large part of their land will probably not make it in the long run. The cost of equipment, fuel and inputs often favors large farms who are able to by wholesale or at a bulk discount, and the expansion of some farms at the expense of others may be causing some tension in rural communities. Farmers also said that it is increasingly difficult to find skilled labor both for peak work periods and to help manage the farm. Many also mentioned that the broadening scope of government regulations and paperwork requirements are negatively affecting farm businesses. A trend in other agricultural sectors such as chicken farming, potatoes or specialty produce is to contract with large companies so that some of the market uncertainty and risk is reduced. This was a distasteful option for many farmers who value their independence. They also felt that corporations are unlikely to want to contract with dryland farmers because of the inherent unpredictability of dryland production, but that corporations may invest in land, which they will then rent to farmers so that farmers still have to deal with all the risk.

8.2.2 Federal commodity and conservation policies

There was a certain amount of bitterness about US farm policy, with the recognition that subsidies for production are often necessary to keep farm businesses

afloat, but also a sense that the USDA and congress no longer truly support family farms. The urban public may not realize that family farms can be 2000-5000 acres, not just the very small farms that market directly to consumers. One grower said that when he started farming in 1980, the USDA seemed very friendly to farmers, but now when he goes to the Farm Service Agency (FSA) or the Administration in Washington D.C., they aren't so interested in helping and seem more like an enemy of the farmer. Another farmer felt that Congress only says it wants to save the family farm, but puts no real effort into defining and protecting family farming. The policy of free trade and cheap food was often criticized, with many farmers feeling that the government and the public are not interested in domestic production or food security, but instead want production wherever the cost is lowest even if this means importing a large percentage of our food supply. Several people at the listening session brought up free trade and stated that this does not consider human rights, the environment or families; that U.S. policy is suppressing farmers and using them as policy pawns. The general sense was that if policies do not change to support farm families there will be far fewer farmers in the near future because many of these more global issues affect prices and opportunities for farmers, but are out of their control.

The USDA conservation reserve program (CRP) is the largest conservation program available in Eastern Washington. A small number of growers also get funds for conservation practices through the environmental quality incentive program (EQIP) and the conservation security program (CSP). The Pacific Northwest Direct

Seed association also supports growers with information and technical resources for no-till practices. At the listening session for the Farm Bill, several growers pointed out that transitioning to conservation practices such as no-till is expensive, and the availability of federal support is quite limited. Many supported the CSP, which makes payments based on addressing conservation issues on the farm before enrollment and then helps farmers make additional changes to improve their environmental stewardship. However, the administration of CSP was criticized as unfair to growers who were not in selected watersheds and burdensome to those in selected watersheds because of paperwork and confusing rules.

A few farmers stated that environmental restrictions were based on junk science or were unscientific and these regulations were hurting their farming operations. While most farmers are very concerned about soil erosion, other environmental issues such as wildlife and water quality are not often discussed. This may be because of the ongoing controversy over salmon migratory routes and the dam system in Eastern Washington, which has put farmers and conservation groups at odds. The CRP has also been controversial, and many growers see it as one reason for declining rural communities. Allowing growers to put their whole farm in CRP has become a retirement plan for some, and if farmers move out of the area after enrolling their farm in CRP, there are fewer dollars being spent in the community for both agricultural and non-agricultural products and services. However, most farmers agreed that the reasons for the decline of rural communities are much larger than

CRP and recognized that CRP could have important environmental benefits if used in a targeted fashion.

8.2.3 Wheat quality and marketing issues

Marketing and quality were key concerns in all of the roundtable discussions. Because most of the wheat grown in Eastern Washington is exported as a commodity, oversees market demands and the marketing systems of countries such as Canada and Australia are very relevant to growers in the region. The Canadian and Australian wheat boards came up several times in the roundtable discussions, because these entities restrict the varieties that growers are allowed to plant to maintain high quality standards in wheat that is bulked as a commodity for export. While growers did not want an agency with the legal power to tell them what varieties they could and could not grow, there was considerable interest in a voluntary system where there would be approved varieties for export under a quality label, or even economic penalties for growing wheat varieties not on the approved list, such as price docking or restrictions on government subsidies. The emphasis was on grower control of the marketing entity, with the ability to market certain varieties based on end-user quality specifications rather than a generic commodity that large companies will buy and blend down to minimum specifications to increase their own profit margins. There are currently only four large companies in the region that ship overseas, but increasing the number of companies would not necessarily increase the price farmers

receive, because the largest ones set the trend and others follow. What is really needed is more grower control over the process. The farmers knew that they could grow high quality wheat, especially through planting varieties appropriate to their geographic location, but when they sell wheat as a generic commodity in a certain market class they lose control of the quality of the product the end user receives.

Alternatives to the commodity market were discussed at length, with many growers feeling that the future of their farm business would depend on being able to differentiate their product in a way that enables them to capture a premium for higher quality. For many of the growers present, direct marketing is not an option because of the large volume of wheat they produce. Niche marketing was appealing, but they were cautious because they recognized that niche markets are often limited and very easy to flood if too many people try to take advantage of them. Expanding domestic markets was a priority, but so was expanding export markets for identity preserved wheat, especially for specific varieties grown in specific conditions to ensure consistent quality. The lower rainfall areas of the state produce high quality high protein (hard red or white) bread wheat while the higher rainfall areas can produce high quality pastry wheat (soft white). Because of the price difference between hard and soft wheat, many growers in the high-rainfall areas are planting hard wheat, even though they know it will be difficult to get a high enough protein concentration. This worries the growers in the low-rainfall zones because it could lower the quality of hard wheat exported generically from the Pacific Northwest.

Growers in the high rainfall zones are also worried because they know they may get docked for low protein, but are frustrated that they cannot get a good price or adequate government support for soft wheat.

In addition to marketing identity preserved varieties, there was some interest in value added marketing such as a grower cooperative that would mill wheat into flour for specific uses or even sell a finished product such as bread, pastries or noodles. This was considered to be considerably more risky because of high transportation costs and because the product would still have to be differentiated at the retail level to be successful. There is one grower coop called Shepherd's Grain that successfully markets flour and baked goods locally with a bakery in Spokane and with the WSU dining services, but many of the growers at the roundtables felt that the niche market for local grains in this region may already be filled. One grower was in the process of starting an organic bakery on his property, but would be sourcing organic wheat from outside the state because organic wheat was not available locally and making the transition to growing his own organic wheat at the same time as starting a retail business was understandably too risky. A different form of value added marketing through wheat that was genetically modified to produce pharmaceuticals, neutraceuticals, or industrial chemicals was also discussed, but there was less interest in this avenue because of market uncertainty.

8.2.4 Farm management and technology

Growers are very aware of the technology treadmill, and the interest in alternative marketing strategies demonstrates a desire to get away from the treadmill of trying to perpetually increase production using new (often expensive) technologies in order to stay afloat with an undifferentiated commodity. There are limits to the economies of scale that can be realized on a family farm. Growers may have to farm more acres to stay in business, and at first this increases the return on their investment in equipment, but once they reach a certain point, they have to buy more equipment and hire additional labor to manage their acres. Expanding acreage does not necessarily increase income in direct proportion to the number of additional acres farmed, but it does directly increase the number of hours each day that one is driving a tractor. Yield was an important factor in their production systems, but there was general agreement that increases in yield do nothing to address the underlying causes of perpetually low commodity prices.

Especially in the low rainfall areas, the farmers were most interested in reducing the amount of inputs needed to produce a high quality crop, and wanted yield stability and drought tolerance rather than maximum yield. Specifically, many growers cited a need to reduce the amount of fertilizer they had to apply and the amount of fuel they had to buy. There was some interest in growing biodiesel for on-farm use, and in planting perennial wheat to reduce the amount of operations they had to do each year. On the survey, several growers also mentioned the need for

varieties better adapted to no-till operations to reduce fuel costs and erosion.

Farmers also agreed about the need to diversify, both their crops and their marketing strategies. There are real challenges to diversification, because farmers often have limited capital to invest in changing their operations. Commodity farms tend to have high cash flow but low net profits and banks are reluctant to provide loans for activities they see as unusual or high risk. Landlords may be unwilling to support lessees who want to produce non-commodity crops, so it is easier for farmers who own their land to diversify. Rotational options are limited in this area, but it is possible to grow many different specialty grains, mustards, legumes, hay crops and seed for various grasses. One farmer mentioned that there should be a program to assist farmers in starting alternative businesses and another agreed that looking at the whole farm system was necessary, that the goal should be to sustain farm families and communities rather than to produce specific commodities or to promote specific management practices and technologies. One grower summed up the general attitude about new technologies very well in his response on the survey "We are also reluctant to try very many things that we are not fairly certain will work in our area. We have seen too many farmers go broke and quit farming doing that in the past. Having said all this I will also say that I am still listening."

8.2.5 Genetically modified wheat

One controversial technology is transgenic wheat with resistance to the popular herbicide glyphosate. A non-transgenic wheat with resistance to imidazole exists, and some growers are using this, but no transgenic wheat has yet been commercialized and there is ongoing discussion about glyphosate resistant wheat. On the survey, growers were split almost exactly in half about their support for Monsanto's decision to suspend development of Roundup Ready wheat indefinitely. Supporters of transgenic wheat felt that they will make management easier, reduce input costs and help with weed control in rotations by reducing potential herbicide carry-over effects (glyphosate breaks down quickly so it is unlikely to impact the next crop as some more persistent herbicides do). Those that wanted transgenic wheat were concerned about their ability to stay competitive without it and felt that transgenic wheat could reduce fertilizer and herbicide costs and provide specific end-user benefits.

Some growers wanted to be able to use the technology, but only in response to specific problems to reduce the risk of weeds evolving resistance to glyphosate and to prolong its utility as a management tool. Many growers, both those that wanted transgenic wheat and those that did not, were concerned about losing glyphosate as an effective herbicide. This could occur if transgenic wheat were planted on large acreage and sprayed exclusively with this one herbicide because there would be high selection pressure on weeds for resistance and also a higher risk of the transgenic varieties outcrossing to a species such as goatgrass which is a major weed and could

receive the resistance genes from wheat. Some use glyphosate to control volunteer wheat in rotations and wondered what would be used to control transgenic volunteer wheat.

Those that agreed with the decision cited marketing concerns, especially because Asian countries so far have not accepted transgenic crops and these represent a large proportion of the export market. Some of these growers wanted transgenic herbicide resistant wheat when the market would accept it, and called for greater public education about genetically engineered crops. Others did not want an herbicide resistant wheat to be commercialized, citing concern over unknown environmental and health impacts, liability issues and intellectual property rights that would prevent them from saving seed and require the payment of a technology use license. In short, the comments and discussion about genetically engineered crops touched on many of the issues discussed in society as a whole, and it is impossible to describe a single "farmer" viewpoint on this topic.

8.2.6 Organic agriculture

There were significant differences between the roundtable discussions and the survey comments about organic farming. This may be a result of the sample, because wheat growers invited to the roundtable were more likely to be familiar and interested in the winter wheat breeding program, which has a strong organic and

low-input component. Growers responding to the survey were members of WAWG, which tends to be a more conventional organization, and only two growers were certified organic. Both groups were skeptical of the possibilities for organic agriculture in this area, but growers at the roundtable discussions were more likely to be supportive of the concept of organic production, while still wondering about its technical feasibility.

A few growers were quite hostile to the idea of organic agriculture on the survey, with comments such as "50 million Americans would starve", that organic agriculture products are inferior, a rip off for the consumer, and a marketing ploy. Others considered it too risky, or uneconomical because increased costs would not be offset enough by the price premium. Serious technical difficulties were another reason why many growers did not want to try transitioning to organic. Many growers were concerned over the potential for increased soil erosion due to mechanical weed control methods and were irritated by organic growers who did not adequately control weeds, causing problems for neighboring farms. The lack of rotational crops in dryland areas was also cited as a barrier to successful organic farming. Low moisture limits the use of legume green manures and other soil-building rotations because growers would lose a year of cash crop production by including a crop to increase soil N or organic matter. Because wheat is a relatively low value crop produced by larger operations, it may be more difficult for organic farming to work here, as opposed to where the climate favors smaller more diversified operations that sell higher-value products.

At the roundtable discussions in the higher rainfall area (Whitman County), growers felt that organic production would be worthwhile for fresh produce, but might not be economical for wheat, especially since the consumer benefit from organic wheat is less than from organic produce. Pesticides are used less frequently on wheat and are not as toxic as those used on some fresh fruits and vegetables that need to have a perfect appearance. The main issues with organic wheat production are the lack of economical organic N sources and the challenge of controlling weeds without increasing erosion. In Montana, organic wheat farming has been very successful, but conditions are different than in Eastern Washington. Growers pointed out that in Montana, weed control is less of a problem because of the climate, there are more rotational options and livestock for nitrogen, and growers aren't expecting as high a yield as they are in the Palouse region.

8.2.7 Perennial wheat

On the survey, priorities identified for the perennial wheat breeding program included an acceptable annual yield (one grower said this would be 30 bushels), plants that do well in highly erodible areas and meet conservation program requirements, plants that require very little fertilizer or water, and plants that could be used to produce bioenergy. Growers also brought up several concerns including the ability to control perennial weeds in a stand of perennial wheat, whether it would be difficult to get rid of perennial wheat when rotating crops, and whether it would

be necessary to remove or burn straw. The market class and quality of the grain were important to growers, who want to make sure perennial wheat will meet the same marketing specifications as annual wheat.

Perennial wheat was of greater interest to growers in lower rainfall zones. In these areas, it is seen as a potential way to adopt no-till practices, which have not been successful so far. It is also of interest because of the potential to reduce input costs. Some growers are interested in grazing livestock on perennial wheat after harvest in the fall. Growers would like to see perennial wheat for filter strips along waterways, steep slopes, sandy areas and land now planted to CRP grasses. There seem to be a few very enthusiastic growers and a larger percentage who are willing to try perennial wheat if it is as economically viable as annual wheat.

8.3 Grower interest in participatory wheat breeding

8.3.1 Participatory Plant Breeding

Participatory plant breeding projects would be complementary to our main breeding program. Interested growers could choose to participate, and participating in a breeding project would be especially beneficial to farmers with difficult areas on their farm where no currently available wheat variety seems to work. Our goal is to

take a breeding strategy that has been very successful in developing countries and make it applicable to commercial farming in Eastern Washington, in order to better address the many environmental challenges growers face in this region. Participatory breeding projects would take some effort on the part of the farmer as well as careful attention from us. They are not without risk, but there is much to be gained by conducting selection directly in the target environment, in this case on a particular farm. We are planning to hold workshops for growers to learn more about the methods and to select traits that would be essential for a successful variety on their farm. Participatory breeding projects could also be of interest to FFA students or anyone interested in learning more about genetics and the breeding process.

A case study in participatory plant breeding will include cooperation with Lexi Roach as she breeds her own variety of wheat. Lexi is a sophomore at Kahlotus High School and is developing a wheat variety as a Future Farmers of America (FFA) project. Her grandfather, Jim Moore, is a collaborator with the winter wheat breeding program and she is making selections on his farm. Lexi made crosses and grew out early generation material in the greenhouse and breeding nursery. Currently her variety is planted on a quarter acre on Jim Moores farm. This year will include roguing plants that are susceptible to disease and testing for quality. In future years there will be enough seed to plant with field scale equipment and selection will continue using evolutionary participatory plant breeding methods (Murphy et al., 2005). This will develop a population particularly suited to the

environmental conditions on the Moore farm, and will explore whether evolutionary participatory plant breeding is a viable option in this region.

Bibliography

Murphy K.M., Lammer D., Lyon S.R., Carter B., Jones S.S., 2005. Breeding for organic and low-input farming systems: An evolutionary-participatory breeding method for inbred cereal grains. Renewable Agriculture and Food Systems 20(1), 48–55.

Chapter 9

Conclusions

9.1 Field and greenhouse studies

Because low input and organic agricultural systems have significantly different environmental conditions than conventional systems, conducting research and selection in these systems will help to optimize crop yields and N dynamics in these systems. Ideally, varieties developed for organic systems would have specific adaptation to conditions in particular ecoregions. Nitrogen use efficiency is likely to be important to most low input and organic systems, but may need to be redefined to include both agronomic and ecological concepts of efficiency.

Because plant breeding in the past half century may have produced varieties that are better adapted to high input systems, historic varieties may have useful traits for organic production that could be reintroduced to breeding programs. From

the greenhouse study, it appears that historic varieties have advantages in terms of biomass production and do not necessarily have lower yields than modern varieties. From the field study, the modern conventionally bred genotypes had the best performance in terms of yield, but had relatively low grain %N compared to historic and perennial varieties. There have clearly been agronomic gains in modern varieties that may carry over to organic systems. Combining the best traits from historic germplasm with the agronomic performance of the modern varieties is one goal in breeding for organic systems.

Both the greenhouse and field studies showed that significant genetic variation exists for traits related to NUE. There were also significant interactions between genotypes and the environment, including crossover interactions, and thus selection will be most effective in the target environment. Main effects of location were not significant as often as the main effects of year, so it may be possible to develop target ecoregions where crossover interactions within the region are minimized.

PCA revealed certain patterns in the variables and genotypes assessed, and was able to clearly separate the historic and conventional genotypes in the field, and the perennial and annual genotypes in the greenhouse. There was substantial overlap between the organically bred genotypes and both the historic and conventional categories in the field, reflecting the breeding history of these lines. The correlation matrix among measured variables helps in understanding the relative independence of these variables and could be used to prioritize measurements and eliminate

redundancy. When the PC scores of genotypes are interpretable as desirable trait combinations, these scores can be used as a tool in selection and planning crosses in addition to grouping genetic material and assessing genetic variation.

9.2 Participatory research

Including farmers in the research and breeding process can help insure that varieties are well suited to particular cropping systems and environments. Participatory plant breeding can benefit farmers who are seeking to lower their synthetic inputs for environmental or economic reasons as well as those farmers faced with harsh environmental conditions. Low-input systems are highly heterogeneous so decentralizing selection nurseries may be necessary for the development of varieties with specific adaptation to certain combinations of environmental stresses. The most efficient way to decentralize selection is to work on farms in the target environment, and to recruit interested farmers to help in directing research priorities and in evaluating plant materials.

While much has been written about the potential for participatory plant breeding in developing countries, farmers in developed countries may also want to be more involved in variety development. Based on a survey of wheat growers in Washington, approximately 52% of growers are interested in a participatory wheat breeding program. Many factors are associated with growers desire to be actively

involved in the research process. Farmers are a very diverse group and and will have different reasons for wanting to work with university plant breeders. Fortunately, participatory breeding projects can be sufficiently adaptable to achieve multiple goals depending on farmer needs and objectives for their farm.

Information from the roundtable discussions and from the survey comments has been useful in understanding the context for participatory research projects, and has also been useful to the breeding program in terms of setting objectives that will meet the farmers long-term goals and vision of farming in this region. These discussions have started a dialog among farmers and researchers that will help the winter wheat breeding program remain relevant and successful.

Appendix A

Abbreviations and acronyms used

A N productivity

AGB aboveground biomass

AM arbuscular mycorrhizal

ANCOVA analysis of variance

ANOVA analysis of covariance

C carbon

DAP days after planting

GPD grain protein deviation

GXE genotype by environment interaction

HI harvest index

KCL potassium chloride

LS means least squares means

MRT mean residence time

N nitrogen

$\mathbf{NH_4^+}$ ammonium

$\mathbf{NO_3^-}$ nitrate

N up N uptake (equivalent to total aboveground plant N)

N ut N utilization efficiency

NHI N harvest index

NUE N use efficiency

PC principal component(s)

PCA principal component analysis

PPB participatory plant breeding

RCBD randomized complete block design

SPAD soil plant analysis development meter

SPAD2 SPAD reading taken immediately prior to anthesis in the field

SPAD3 SPAD reading taken immediately post-anthesis in the field

TN Total nitrogen (%N $*$ weight)

WAWG Washington Association of Wheat Growers

Appendix B

Comparisons of LS mean values for greenhouse experiment

genotype	weight (g)	std err	entry	_6	_13	_10	_3	_15	_7	_12	_20	_5	_2	_19	_21	_14	_4	_16	_8	_11	_18	_9	_17
Idaed	3.20	0.278	6	_	0.9996	0.9979	0.9926	0.9698	0.9034	0.7378	0.6212	0.6275	0.5467	0.4397	0.3465	0.1643	0.1659	0.1024	0.1365	0.0434	0.0138	0.0065	0.0046
Sonora	2.71	0.278	13	0.9996	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9996	0.9995	0.9976	0.9937	0.9845	0.9181	0.8994	0.8274	0.8478	0.6381	0.3787	0.2529	0.1272
Pilcraw	2.64	0.281	10	0.9979	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9995	0.9986	0.9952	0.9670	0.9565	0.9181	0.9193	0.7232	0.5142	0.3550	0.1788
Bunyip	2.58	0.282	3	0.9926	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9997	0.9990	0.9861	0.9782	0.9508	0.9598	0.8794	0.6237	0.4860	0.2587
Surprise	2.50	0.280	15	0.9698	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9971	0.9946	0.9852	0.9868	0.9424	0.7635	0.6289	0.3545
Onas	2.28	0.361	7	0.9034	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9996	0.9954	0.9822	0.8281
Scarlet	2.28	0.280	12	0.7378	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9999	0.9986	0.9765	0.9342	0.6883
White Marquis	2.22	0.278	20	0.6212	0.9996	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9997	0.9915	0.9688	0.7704
Currawa	2.20	0.287	5	0.6275	0.9995	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9946	0.9799	0.8120
Arco	2.10	0.322	2	0.5467	0.9976	0.9995	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9998	0.9985	0.9365
White Federation	2.08	0.301	19	0.4397	0.9937	0.9986	0.9997	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9987	0.9396
Zak	2.03	0.300	21	0.3465	0.9845	0.9952	0.9990	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9996	0.9620
Spinkcota	1.93	0.278	14	0.1643	0.9181	0.9670	0.9861	0.9971	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9854
Canus	1.88	0.302	4	0.1659	0.8994	0.9565	0.9782	0.9946	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9956
Wakanz	1.85	0.281	16	0.1024	0.8274	0.9181	0.9508	0.9852	1.0000	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	0.9965
Pacific Bluestem	1.80	0.321	8	0.1365	0.8478	0.9193	0.9598	0.9868	1.0000	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	0.9992
Red Fife	1.72	0.291	11	0.0434	0.6381	0.7232	0.8794	0.9424	0.9996	0.9986	0.9997	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	0.9998
Westbred Express	1.60	0.278	18	0.0138	0.3787	0.5142	0.6237	0.7635	0.9954	0.9765	0.9915	0.9946	0.9998	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000
Penawawa	1.52	0.278	9	0.0065	0.2529	0.3550	0.4860	0.6289	0.9822	0.9342	0.9688	0.9799	0.9985	0.9987	0.9996	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000
Wawawai	1.15	0.377	17	0.0046	0.1272	0.1788	0.2587	0.3545	0.8281	0.6883	0.7704	0.8120	0.9365	0.9396	0.9620	0.9854	0.9956	0.9965	0.9992	0.9998	1.0000	1.0000	_

genotype	%N	std err	entry	_20	_5	_4	_16	_19	_14	_18	_2	_15	_17	_12	_9	_7	_11	_10	_13	_3	_21	_6	_8
White Marquis	5.926	0.1466	20	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	1.0000	0.9976	0.9973	0.9976	0.9850	0.9013	0.8900	0.7263	0.7862	0.3625	0.2283
Currawa	5.914	0.1511	5	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9986	0.9987	0.9988	0.9935	0.9394	0.9231	0.7615	0.8401	0.4379	0.2737
Canus	5.848	0.1591	4	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9996	0.9915	0.9897	0.9436	0.9623	0.7083	0.5126
Wakanz	5.789	0.1483	16	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9988	0.9984	0.9825	0.9900	0.8330	0.6465
White Federation	5.782	0.1588	19	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9994	0.9993	0.9911	0.9945	0.8840	0.7212
Spinkcota	5.771	0.1467	14	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9994	0.9993	0.9906	0.9942	0.8692	0.6983
Westbred Express	5.768	0.1464	18	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9995	0.9994	0.9917	0.9947	0.8735	0.7060
Arco	5.755	0.1696	2	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9999	0.9983	0.9987	0.9483	0.8387
Surprise	5.696	0.1474	15	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9997	0.9998	0.9769	0.8929
Wawawai	5.674	0.1984	17	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9985	0.9838
Scarlet	5.631	0.1474	12	0.9976	0.9986	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9977	0.9732
Penawawa	5.627	0.1465	9	0.9973	0.9987	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9979	0.9755
Onas	5.583	0.1900	7	0.9976	0.9988	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9988
Red Fife	5.570	0.1534	11	0.9850	0.9935	0.9996	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	0.9999	0.9970
Pilcraw	5.502	0.1483	10	0.9013	0.9394	0.9915	0.9988	0.9994	0.9994	0.9995	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	0.9998
Sonora	5.502	0.1463	13	0.8900	0.9231	0.9897	0.9984	0.9993	0.9993	0.9994	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	0.9998
Bunyip	5.441	0.1485	3	0.7263	0.7615	0.9436	0.9825	0.9911	0.9906	0.9917	0.9983	0.9997	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000
Zak	5.440	0.1582	21	0.7862	0.8401	0.9623	0.9900	0.9945	0.9942	0.9947	0.9987	0.9998	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000
Idaed	5.336	0.1465	6	0.3625	0.4379	0.7083	0.8330	0.8840	0.8692	0.8735	0.9483	0.9769	0.9985	0.9977	0.9979	1.0000	0.9999	1.0000	1.0000	1.0000	1.0000	_	1.0000
Pacific Bluestem	5.239	0.1690	8	0.2283	0.2737	0.5126	0.6465	0.7212	0.6983	0.7060	0.8387	0.8929	0.9838	0.9732	0.9755	0.9988	0.9970	0.9998	0.9998	1.0000	1.0000	1.0000	_

Table B.1: Genotype LS mean values and pairwise comparisons for aboveground biomass (weight) and tissue N concentration (%N) for annual genotypes at the 6leaf stage. Significant differences at the p<0.05 level are shaded.

genotype	TN (g)	std err	entry	_6	_15	_10	_13	_3	_20	_12	_7	_5	_19	_2	_14	_4	_16	_21	_8	_11	_18	_9	_17
Idaed	0.160	0.0120	6	_	0.9997	0.9996	0.9990	0.9935	0.9592	0.9274	0.9646	0.8788	0.6825	0.4480	0.2014	0.2417	0.0944	0.1102	0.0511	0.0118	0.0089	0.0001	0.0016
Surprise	0.140	0.0120	15	0.9997	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9996	0.9917	0.9350	0.9423	0.7809	0.8195	0.5937	0.3703	0.2801	0.0169	0.0569
Pilcraw	0.139	0.0121	10	0.9996	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9998	0.9927	0.9520	0.9594	0.8354	0.8333	0.6285	0.3197	0.3173	0.0181	0.0587
Sonora	0.138	0.0120	13	0.9990	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9960	0.9640	0.9688	0.8545	0.8694	0.6689	0.4031	0.3487	0.0226	0.0706
Bunyip	0.134	0.0121	3	0.9935	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9996	0.9915	0.9921	0.9378	0.9535	0.8138	0.6281	0.5151	0.0523	0.1288
White Marquis	0.129	0.0120	20	0.9592	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9992	0.9992	0.9871	0.9894	0.9242	0.7742	0.7078	0.1023	0.2098
Scarlet	0.127	0.0121	12	0.9274	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	0.9998	0.9998	0.9949	0.9960	0.9576	0.8566	0.7933	0.1464	0.2698
Onas	0.125	0.0155	7	0.9646	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9998	0.9998	0.9947	0.9636	0.9606	0.4337	0.5204
Currawa	0.125	0.0123	5	0.8788	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	0.9988	0.9992	0.9836	0.9388	0.8867	0.2359	0.3728
White Federation	0.118	0.0130	19	0.6825	0.9996	0.9998	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9994	0.9936	0.9886	0.5247	0.6354
Arco	0.111	0.0139	2	0.4480	0.9917	0.9927	0.9960	0.9996	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9999	0.8466	0.8772
Spinkcota	0.107	0.0120	14	0.2014	0.9350	0.9520	0.9640	0.9915	0.9992	0.9998	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.8661	0.9028
Canus	0.106	0.0130	4	0.2417	0.9423	0.9594	0.9688	0.9921	0.9992	0.9998	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9222	0.9380
Wakanz	0.102	0.0121	16	0.0944	0.7809	0.8354	0.8545	0.9378	0.9871	0.9949	0.9998	0.9988	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	0.9726	0.9769
Zak	0.101	0.0129	21	0.1102	0.8195	0.8333	0.8694	0.9535	0.9894	0.9960	0.9998	0.9992	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	0.9840	0.9846
Pacific Bluestem	0.094	0.0138	8	0.0511	0.5937	0.6285	0.6689	0.8138	0.9242	0.9576	0.9947	0.9836	0.9994	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	0.9998	0.9995
Red Fife	0.090	0.0125	11	0.0118	0.3703	0.3197	0.4031	0.6281	0.7742	0.8566	0.9636	0.9388	0.9936	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	0.9999
Westbred Express	0.089	0.0120	18	0.0089	0.2801	0.3173	0.3487	0.5151	0.7078	0.7933	0.9606	0.8867	0.9886	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	0.9999
Penawawa	0.072	0.0120	9	0.0001	0.0169	0.0181	0.0226	0.0523	0.1023	0.1464	0.4337	0.2359	0.5247	0.8466	0.8661	0.9222	0.9726	0.9840	0.9998	1.0000	1.0000	_	1.0000
Wawawai	0.067	0.0162	17	0.0016	0.0569	0.0587	0.0706	0.1288	0.2098	0.2698	0.5204	0.3728	0.6354	0.8772	0.9028	0.9380	0.9769	0.9846	0.9995	0.9999	0.9999	1.0000	_

Table B.2: Genotype LS mean values and pairwise comparisons for total plant N (TN) for annual genotypes at the 6leaf stage. Significant differences at the p<0.05 level are shaded.

genotype	weight (g)	std err	entry	_8	_2	_10	_3	_7	_13	_18	_6	_14	_11	_4	_20	_9	_5	_16	_12	_15	_19	_21
Pacific Bluestem	45.96	2.5853	8	_	1.0000	0.8590	0.7213	0.6399	0.5394	0.1411	0.0349	0.0147	0.0048	0.0054	0.0101	0.0028	0.0033	0.0004	0.0002	0.0011	<.0001	<.0001
Arco	45.76	2.4691	2	1.0000	_	0.8602	0.7240	0.6376	0.5339	0.1274	0.0318	0.0134	0.0041	0.0046	0.0091	0.0023	0.0029	0.0003	0.0001	0.0010	<.0001	<.0001
Pilcraw	39.22	2.1388	10	0.8590	0.8602	_	1.0000	1.0000	1.0000	0.9992	0.8730	0.6090	0.4495	0.4505	0.5312	0.2925	0.2440	0.1204	0.0893	0.1050	0.0029	0.0009
Bunyip	38.14	2.1429	3	0.7213	0.7240	1.0000	_	1.0000	1.0000	1.0000	0.9735	0.8139	0.6971	0.6923	0.7520	0.5065	0.4145	0.2770	0.2305	0.2017	0.0071	0.0029
Onas	37.73	2.3040	7	0.6399	0.6376	1.0000	1.0000	_	1.0000	1.0000	0.9932	0.9102	0.8382	0.8372	0.8757	0.6884	0.6126	0.4262	0.3564	0.3226	0.0232	0.0086
Sonora	37.48	2.1319	13	0.5394	0.5339	1.0000	1.0000	1.0000	_	1.0000	0.9942	0.9126	0.8361	0.8344	0.8756	0.6766	0.5939	0.4091	0.3412	0.3096	0.0174	0.0065
Westbred Express	35.30	2.3797	18	0.1411	0.1274	0.9992	1.0000	1.0000	1.0000	_	1.0000	0.9996	0.9987	0.9988	0.9993	0.9913	0.9835	0.9257	0.8780	0.8250	0.2557	0.1092
Idaed	33.08	2.1484	6	0.0349	0.0318	0.8730	0.9735	0.9932	0.9942	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9991	0.9976	0.9807	0.5205	0.3207
Spinkcota	31.20	2.4833	14	0.0147	0.0134	0.6090	0.8139	0.9102	0.9126	0.9996	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9497	0.8357
Red Fife	31.16	2.1408	11	0.0048	0.0041	0.4495	0.6971	0.8382	0.8361	0.9987	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9130	0.7494
Canus	31.14	2.1488	4	0.0054	0.0046	0.4505	0.6923	0.8372	0.8344	0.9988	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9134	0.7541
White Marquis	31.12	2.3341	20	0.0101	0.0091	0.5312	0.7520	0.8757	0.8756	0.9993	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	0.9999	0.9371	0.8084
Penawawa	30.37	2.1592	9	0.0028	0.0023	0.2925	0.5065	0.6884	0.6766	0.9913	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	0.9999	0.9751	0.8881
Currawa	29.91	2.2255	5	0.0033	0.0029	0.2440	0.4145	0.6126	0.5939	0.9835	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	0.9903	0.9440
Wakanz	29.30	2.1320	16	0.0004	0.0003	0.1204	0.2770	0.4262	0.4091	0.9257	0.9991	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	0.9986	0.9789
Scarlet	28.96	2.1436	12	0.0002	0.0001	0.0893	0.2305	0.3564	0.3412	0.8780	0.9976	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	0.9996	0.9902
Surprise	27.43	2.7839	15	0.0011	0.0010	0.1050	0.2017	0.3226	0.3096	0.8250	0.9807	0.9999	0.9999	0.9999	0.9999	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000
White Federation	25.32	2.2212	19	<.0001	<.0001	0.0029	0.0071	0.0232	0.0174	0.2557	0.5205	0.9497	0.9130	0.9134	0.9371	0.9751	0.9903	0.9986	0.9996	1.0000	_	1.0000
Zak	24.15	2.3115	21	<.0001	<.0001	0.0009	0.0029	0.0086	0.0065	0.1092	0.3207	0.8357	0.7494	0.7541	0.8084	0.8881	0.9440	0.9789	0.9902	1.0000	1.0000	_

genotype	%N	std err	entry	_21	_5	_9	_19	_6	_4	_16	_12	_11	_20	_14	_7	_18	_15	_8	_10	_13	_3	_2
Zak	1.806	0.0934	21	_	1.0000	1.0000	0.9997	0.8601	0.8307	0.6477	0.3809	0.0981	0.0329	0.0314	0.0074	0.0090	0.0153	0.0002	<.0001	<.0001	<.0001	<.0001
Currawa	1.721	0.0900	5	1.0000	_	1.0000	1.0000	0.9956	0.9930	0.9610	0.8260	0.3666	0.1449	0.1370	0.0497	0.0692	0.0673	0.0026	<.0001	<.0001	<.0001	0.0003
Penawawa	1.698	0.0873	9	1.0000	1.0000	_	1.0000	0.9991	0.9984	0.9840	0.8901	0.4742	0.2126	0.1972	0.0688	0.0826	0.1017	0.0024	0.0001	<.0001	<.0001	0.0003
White Federation	1.661	0.0898	19	0.9997	1.0000	1.0000	_	1.0000	1.0000	0.9988	0.9786	0.7117	0.3864	0.3596	0.1664	0.2058	0.1969	0.0108	0.0006	0.0004	0.0003	0.0016
Idaed	1.547	0.0868	6	0.8601	0.9956	0.9991	1.0000	_	1.0000	1.0000	1.0000	0.9969	0.9348	0.9088	0.6939	0.7063	0.7246	0.0748	0.0119	0.0103	0.0095	0.0140
Canus	1.539	0.0869	4	0.8307	0.9930	0.9984	1.0000	1.0000	_	1.0000	1.0000	0.9982	0.9503	0.9279	0.7330	0.7439	0.7585	0.0866	0.0145	0.0126	0.0117	0.0169
Wakanz	1.503	0.0862	16	0.6477	0.9610	0.9840	0.9988	1.0000	1.0000	_	1.0000	0.9999	0.9920	0.9848	0.8862	0.8771	0.9015	0.1305	0.0336	0.0317	0.0321	0.0258
Scarlet	1.453	0.0866	12	0.3809	0.8260	0.8901	0.9786	1.0000	1.0000	1.0000	_	1.0000	0.9998	0.9994	0.9839	0.9788	0.9848	0.2638	0.1053	0.1037	0.1088	0.0634
Red Fife	1.379	0.0865	11	0.0981	0.3666	0.4742	0.7117	0.9969	0.9982	0.9999	1.0000	_	1.0000	1.0000	1.0000	0.9999	0.9999	0.7182	0.4115	0.3915	0.3831	0.3459
White Marquis	1.312	0.0943	20	0.0329	0.1449	0.2126	0.3864	0.9348	0.9503	0.9920	0.9998	1.0000	_	1.0000	1.0000	1.0000	1.0000	0.9686	0.8511	0.8350	0.8248	0.7820
Spinkcota	1.293	0.1004	14	0.0314	0.1370	0.1972	0.3596	0.9088	0.9279	0.9848	0.9994	1.0000	1.0000	_	1.0000	1.0000	1.0000	0.9887	0.9350	0.9269	0.9221	0.8774
Onas	1.251	0.0931	7	0.0074	0.0497	0.0688	0.1664	0.6939	0.7330	0.8862	0.9839	1.0000	1.0000	1.0000	_	1.0000	1.0000	0.9974	0.9820	0.9803	0.9803	0.9332
Westbred Express	1.244	0.0962	18	0.0090	0.0692	0.0826	0.2058	0.7063	0.7439	0.8771	0.9788	0.9999	1.0000	1.0000	1.0000	_	1.0000	0.9974	0.9885	0.9885	0.9897	0.9235
Surprise	1.226	0.1125	15	0.0153	0.0673	0.1017	0.1969	0.7246	0.7585	0.9015	0.9848	0.9999	1.0000	1.0000	1.0000	1.0000	_	0.9999	0.9989	0.9987	0.9985	0.9941
Pacific Bluestem	1.063	0.1045	8	0.0002	0.0026	0.0024	0.0108	0.0748	0.0866	0.1305	0.2638	0.7182	0.9686	0.9887	0.9974	0.9974	0.9999	_	1.0000	1.0000	1.0000	1.0000
Pilcraw	1.046	0.0865	10	<.0001	<.0001	0.0001	0.0006	0.0119	0.0145	0.0336	0.1053	0.4115	0.8511	0.9350	0.9820	0.9885	0.9989	1.0000	_	1.0000	1.0000	1.0000
Sonora	1.044	0.0862	13	<.0001	<.0001	<.0001	0.0004	0.0103	0.0126	0.0317	0.1037	0.3915	0.8350	0.9269	0.9803	0.9885	0.9987	1.0000	1.0000	_	1.0000	1.0000
Bunyip	1.044	0.0866	3	<.0001	<.0001	<.0001	0.0003	0.0095	0.0117	0.0321	0.1088	0.3831	0.8248	0.9221	0.9803	0.9897	0.9985	1.0000	1.0000	1.0000	_	1.0000
Arco	1.000	0.0998	2	<.0001	0.0003	0.0003	0.0016	0.0140	0.0169	0.0258	0.0634	0.3459	0.7820	0.8774	0.9332	0.9235	0.9941	1.0000	1.0000	1.0000	1.0000	_

Table B.3: Genotype LS mean values and pairwise comparisons for aboveground biomass (weight), tissue N concentration (%N) and total plant N (TN) for annual genotypes at anthesis. Significant differences at the p<0.05 level are shaded.

genotype	TN (g)	std err	entry	_6	_7	_9	_5	_4	_8	_18	_12	_10	_2	_16	_3	_21	_20	_13	_14	_11	_19	_15
Idaed	0.4927	0.02183	6	_	1.0000	1.0000	0.9892	0.9683	0.9778	0.6522	0.5106	0.3787	0.2415	0.1549	0.1139	0.1064	0.0709	0.0332	0.0619	0.0262	0.0156	0.0630
Onas	0.4716	0.02358	7	1.0000	_	1.0000	1.0000	1.0000	1.0000	0.9763	0.9458	0.8835	0.7632	0.6332	0.5455	0.5023	0.3993	0.2672	0.3486	0.2292	0.1618	0.3206
Penawawa	0.4656	0.02183	9	1.0000	1.0000	_	1.0000	1.0000	1.0000	0.9894	0.9712	0.9265	0.8251	0.7020	0.6134	0.5686	0.4582	0.3100	0.4028	0.2665	0.1885	0.3720
Currawa	0.4453	0.02183	5	0.9892	1.0000	1.0000	_	1.0000	1.0000	1.0000	0.9999	0.9993	0.9942	0.9775	0.9554	0.9325	0.8756	0.7677	0.8243	0.7179	0.6038	0.7743
Canus	0.4400	0.02183	4	0.9683	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	0.9999	0.9987	0.9930	0.9833	0.9708	0.9369	0.8635	0.8998	0.8245	0.7261	0.8575
Pacific Bluestem	0.4398	0.02358	8	0.9778	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	0.9993	0.9957	0.9895	0.9803	0.9554	0.9006	0.9254	0.8693	0.7865	0.8876
Westbred Express	0.4163	0.02358	18	0.6522	0.9763	0.9894	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9993	0.9994	0.9986	0.9944	0.9977
Scarlet	0.4134	0.02183	12	0.5106	0.9458	0.9712	0.9999	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9996	0.9997	0.9991	0.9959	0.9984
Pilcraw	0.4076	0.02183	10	0.3787	0.8835	0.9265	0.9993	0.9999	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9993	0.9997
Arco	0.4004	0.02183	2	0.2415	0.7632	0.8251	0.9942	0.9987	0.9993	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
Wakanz	0.3944	0.02183	16	0.1549	0.6332	0.7020	0.9775	0.9930	0.9957	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
Bunyip	0.3905	0.02183	3	0.1139	0.5455	0.6134	0.9554	0.9833	0.9895	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
Zak	0.3855	0.02358	21	0.1064	0.5023	0.5686	0.9325	0.9708	0.9803	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
White Marquis	0.3807	0.02358	20	0.0709	0.3993	0.4582	0.8756	0.9369	0.9554	0.9999	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000
Sonora	0.3771	0.02183	13	0.0332	0.2672	0.3100	0.7677	0.8635	0.9006	0.9993	0.9996	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000
Spinkcota	0.3748	0.02521	14	0.0619	0.3486	0.4028	0.8243	0.8998	0.9254	0.9994	0.9997	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000
Red Fife	0.3748	0.02183	11	0.0262	0.2292	0.2665	0.7179	0.8245	0.8693	0.9986	0.9991	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000
White Federation	0.3698	0.02183	19	0.0156	0.1618	0.1885	0.6038	0.7261	0.7865	0.9944	0.9959	0.9993	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000
Surprise	0.3669	0.02819	15	0.0630	0.3206	0.3720	0.7743	0.8575	0.8876	0.9977	0.9984	0.9997	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_

Table B.4: Genotype LS mean values and pairwise comparisons for total plant N (TN) for annual genotypes at anthesis. Significant differences at the $p<0.05$ level are shaded.

genotype	grain wt	std err	entry	_17	_7	_9	_3	_21	_10	_19	_4	_20	_6	_14	_1	_5	_2	_15	_16	_12	_18	_11	_13	_8
Wawawai	26.6717	2.011	**17**	_	1.0000	1.0000	1.0000	0.9997	0.9937	0.9755	0.8720	0.8153	0.5524	0.4283	0.6736	0.4488	0.4896	0.4092	0.3283	0.1145	0.0381	0.0243	0.0199	0.0020
Onas	24.5978	1.881	**7**	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9987	0.9964	0.9509	0.8932	0.9666	0.8963	0.9109	0.8710	0.8166	0.4779	0.2251	0.1599	0.1367	0.0187
Penawawa	24.5189	1.741	**9**	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	0.9984	0.9954	0.9392	0.8705	0.9617	0.8765	0.8951	0.8472	0.7823	0.4222	0.1820	0.1248	0.1050	0.0125
Bunyip	23.9633	1.741	**3**	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	0.9999	0.9995	0.9825	0.9507	0.9879	0.9517	0.9597	0.9355	0.8987	0.5887	0.2968	0.2144	0.1843	0.0257
Zak	23.3361	1.881	**21**	0.9997	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	0.9985	0.9933	0.9985	0.9927	0.9938	0.9889	0.9802	0.8224	0.5455	0.4366	0.3920	0.0795
Pilcraw	22.5767	1.741	**10**	0.9937	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	0.9999	0.9993	0.9999	0.9991	0.9993	0.9984	0.9966	0.9215	0.6952	0.5822	0.5324	0.1234
White Federation	22.0500	1.741	**19**	0.9755	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	0.9999	1.0000	0.9999	0.9999	0.9998	0.9996	0.9734	0.8308	0.7365	0.6907	0.2029
Canus	21.1178	1.741	**4**	0.8720	0.9987	0.9984	0.9999	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9985	0.9668	0.9284	0.9049	0.4174
White Marquis	20.8278	1.741	**20**	0.8153	0.9964	0.9954	0.9995	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9996	0.9836	0.9596	0.9436	0.4993
Idaed	19.8178	1.741	**6**	0.5524	0.9509	0.9392	0.9825	0.9985	0.9999	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9995	0.9978	0.9960	0.7815
Spinkcota	19.3689	1.741	**14**	0.4283	0.8932	0.8705	0.9507	0.9933	0.9993	0.9999	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9997	0.9993	0.8764
Alpowa	19.1933	2.358	**1**	0.6736	0.9666	0.9617	0.9879	0.9985	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9795
Currawa	19.1917	1.881	**5**	0.4488	0.8963	0.8765	0.9517	0.9927	0.9991	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9310
Arco	19.1050	2.011	**2**	0.4896	0.9109	0.8951	0.9597	0.9938	0.9993	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9569
Surprise	19.0378	1.881	**15**	0.4092	0.8710	0.8472	0.9355	0.9889	0.9984	0.9998	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	0.9999	0.9475
Wakanz	18.9722	1.741	**16**	0.3283	0.8166	0.7823	0.8987	0.9802	0.9966	0.9996	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	0.9999	0.9348
Scarlet	17.7367	1.741	**12**	0.1145	0.4779	0.4222	0.5887	0.8224	0.9215	0.9734	0.9985	0.9996	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	0.9972
Westbred Express	16.7078	1.741	**18**	0.0381	0.2251	0.1820	0.2968	0.5455	0.6952	0.8308	0.9668	0.9836	0.9995	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000
Red Fife	16.3289	1.741	**11**	0.0243	0.1599	0.1248	0.2144	0.4366	0.5822	0.7365	0.9284	0.9596	0.9978	0.9997	1.0000	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000
Sonora	16.1656	1.741	**13**	0.0199	0.1367	0.1050	0.1843	0.3920	0.5324	0.6907	0.9049	0.9436	0.9960	0.9993	1.0000	0.9998	0.9999	0.9999	0.9999	1.0000	1.0000	1.0000	_	1.0000
Pacific Bluestem	14.0411	1.881	**8**	0.0020	0.0187	0.0125	0.0257	0.0795	0.1234	0.2029	0.4174	0.4993	0.7815	0.8764	0.9795	0.9310	0.9569	0.9475	0.9348	0.9972	1.0000	1.0000	1.0000	_

genotype	grain %N	std err	entry	_18	_8	_13	_6	_5	_11	_19	_12	_14	_20	_16	_1	_4	_2	_15	_21	_3	_7	_9	_10	_17
Westbred Express	2.444	0.12305	**18**	_	1.0000	1.0000	0.9996	0.9985	0.8446	0.8254	0.8163	0.8153	0.6531	0.1523	0.4407	0.1185	0.1881	0.0890	0.0142	0.0064	0.0114	0.0034	0.0006	0.0008
Pacific Bluestem	2.285	0.13291	**8**	1.0000	_	1.0000	1.0000	1.0000	0.9998	0.9997	0.9997	0.9997	0.9965	0.7890	0.9401	0.7271	0.7937	0.6228	0.2354	0.1564	0.2048	0.1029	0.0307	0.0278
Sonora	2.282	0.12305	**13**	1.0000	1.0000	_	1.0000	1.0000	0.9997	0.9996	0.9995	0.9995	0.9949	0.7406	0.9274	0.6712	0.7536	0.5642	0.1881	0.1168	0.1611	0.0735	0.0196	0.0188
Idaed	2.228	0.12305	**6**	0.9996	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9998	0.9067	0.9822	0.8636	0.9054	0.7761	0.3510	0.2443	0.3100	0.1656	0.0518	0.0467
Currawa	2.195	0.13291	**5**	0.9985	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9740	0.9959	0.9559	0.9697	0.9063	0.5484	0.4309	0.5014	0.3211	0.1262	0.1064
Red Fife	2.071	0.12305	**11**	0.8446	0.9998	0.9997	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	0.9999	1.0000	0.9998	0.9998	0.9982	0.9124	0.8480	0.8863	0.7466	0.4319	0.3618
White Federation	2.064	0.12305	**19**	0.8254	0.9997	0.9996	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9999	0.9987	0.9245	0.8657	0.9006	0.7698	0.4576	0.3840
Scarlet	2.062	0.12305	**12**	0.8163	0.9997	0.9995	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	0.9999	0.9999	0.9989	0.9295	0.8734	0.9067	0.7800	0.4694	0.3942
Spinkcota	2.061	0.12305	**14**	0.8153	0.9997	0.9995	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	0.9999	0.9999	0.9989	0.9300	0.8741	0.9073	0.7810	0.4706	0.3952
White Marquis	2.019	0.12305	**20**	0.6531	0.9965	0.9949	0.9998	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	0.9999	0.9800	0.9570	0.9705	0.9053	0.6527	0.5602
Wakanz	1.879	0.12305	**16**	0.1523	0.7890	0.7406	0.9067	0.9740	0.9999	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9998	0.9871	0.9607
Alpowa	1.879	0.16661	**1**	0.4407	0.9401	0.9274	0.9822	0.9959	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9985	0.9924
Canus	1.861	0.12305	**4**	0.1185	0.7271	0.6712	0.8636	0.9559	0.9998	0.9999	0.9999	0.9999	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9937	0.9767
Arco	1.850	0.14209	**2**	0.1881	0.7937	0.7536	0.9054	0.9697	0.9998	0.9999	0.9999	0.9999	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9986	0.9925
Surprise	1.818	0.13291	**15**	0.0890	0.6228	0.5642	0.7761	0.9063	0.9982	0.9987	0.9989	0.9989	0.9999	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	0.9996	0.9970
Zak	1.710	0.13291	**21**	0.0142	0.2354	0.1881	0.3510	0.5484	0.9124	0.9245	0.9295	0.9300	0.9800	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000
Bunyip	1.699	0.12305	**3**	0.0064	0.1564	0.1168	0.2443	0.4309	0.8480	0.8657	0.8734	0.8741	0.9570	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000
Onas	1.698	0.13291	**7**	0.0114	0.2048	0.1611	0.3100	0.5014	0.8863	0.9006	0.9067	0.9073	0.9705	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000
Penawawa	1.669	0.12305	**9**	0.0034	0.1029	0.0735	0.1656	0.3211	0.7466	0.7698	0.7800	0.7810	0.9053	0.9998	1.0000	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000
Pilcraw	1.594	0.12305	**10**	0.0006	0.0307	0.0196	0.0518	0.1262	0.4319	0.4576	0.4694	0.4706	0.6527	0.9871	0.9985	0.9937	0.9986	0.9996	1.0000	1.0000	1.0000	1.0000	_	1.0000
Wawawai	1.536	0.14209	**17**	0.0008	0.0278	0.0188	0.0467	0.1064	0.3618	0.3840	0.3942	0.3952	0.5602	0.9607	0.9924	0.9767	0.9925	0.9970	1.0000	1.0000	1.0000	1.0000	1.0000	_

Table B.5: Genotype LS mean values and pairwise comparisons for grain weight and grain N concentration for annual genotypes at maturity. Significant differences at the p<0.05 level are shaded.

genotype	grain TN	std err	entry	_19	_6	_20	_5	_17	_7	_3	_14	_4	_21	_9	_18	_15	_12	_11	_10	_2	_1	_13	_16	_8
White Federation	0.4493	0.02489	**19**	_	1.0000	1.0000	1.0000	1.0000	0.9999	0.9998	0.9995	0.9994	0.9994	0.9657	0.9153	0.8862	0.6677	0.6471	0.6223	0.5149	0.5933	0.1938	0.1544	0.0035
Idaed	0.4358	0.02489	**6**	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9978	0.9901	0.9820	0.8943	0.8822	0.8667	0.7705	0.8139	0.4177	0.3526	0.0129
White Marquis	0.4355	0.02489	**20**	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9980	0.9907	0.9828	0.8973	0.8854	0.8702	0.7748	0.8174	0.4229	0.3574	0.0133
Currawa	0.4219	0.02688	**5**	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9995	0.9916	0.9897	0.9871	0.9567	0.9622	0.7700	0.7084	0.0655
Wawawai	0.4115	0.02873	**17**	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9996	0.9995	0.9992	0.9942	0.9940	0.9365	0.9064	0.1785
Onas	0.4085	0.02688	**7**	0.9999	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9997	0.9996	0.9995	0.9954	0.9952	0.9399	0.9096	0.1660
Bunyip	0.4070	0.02489	**3**	0.9998	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9997	0.9996	0.9994	0.9948	0.9949	0.9289	0.8937	0.1359
Spinkcota	0.4048	0.02489	**14**	0.9995	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9998	0.9997	0.9968	0.9967	0.9467	0.9172	0.1571
Canus	0.4042	0.02489	**4**	0.9994	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	0.9999	0.9998	0.9998	0.9972	0.9971	0.9511	0.9233	0.1637
Zak	0.4023	0.02688	**21**	0.9994	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9989	0.9987	0.9754	0.9587	0.2398
Penawawa	0.3861	0.02489	**9**	0.9657	0.9978	0.9980	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9988	0.9971	0.4461
Westbred Express	0.3795	0.02489	**18**	0.9153	0.9901	0.9907	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9996	0.5788
Surprise	0.3740	0.02688	**15**	0.8862	0.9820	0.9828	0.9995	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.7497
Scarlet	0.3641	0.02489	**12**	0.6677	0.8943	0.8973	0.9916	0.9996	0.9997	0.9997	0.9999	0.9999	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.8564
Red Fife	0.3631	0.02489	**11**	0.6471	0.8822	0.8854	0.9897	0.9995	0.9996	0.9996	0.9998	0.9998	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.8696
Pilcraw	0.3619	0.02489	**10**	0.6223	0.8667	0.8702	0.9871	0.9992	0.9995	0.9994	0.9997	0.9998	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	0.8843
Arco	0.3494	0.02873	**2**	0.5149	0.7705	0.7748	0.9567	0.9942	0.9954	0.9948	0.9968	0.9972	0.9989	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	0.9899
Alpowa	0.3436	0.03369	**1**	0.5933	0.8139	0.8174	0.9622	0.9940	0.9952	0.9949	0.9967	0.9971	0.9987	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	0.9991
Sonora	0.3386	0.02489	**13**	0.1938	0.4177	0.4229	0.7700	0.9365	0.9399	0.9289	0.9467	0.9511	0.9754	0.9988	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	0.9976
Wakanz	0.3352	0.02489	**16**	0.1544	0.3526	0.3574	0.7084	0.9064	0.9096	0.8937	0.9172	0.9233	0.9587	0.9971	0.9996	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	0.9990
Pacific Bluestem	0.2864	0.02688	**8**	0.0035	0.0129	0.0133	0.0655	0.1785	0.1660	0.1359	0.1571	0.1637	0.2398	0.4461	0.5788	0.7497	0.8564	0.8696	0.8843	0.9899	0.9991	0.9976	0.9990	_

genotype	straw wt (g)	std err	entry	_8	_10	_2	_4	_14	_3	_20	_7	_15	_13	_9	_21	_17	_11	_16	_19	_5	_6	_1	_18	_12
Pacific Bluestem	43.5211	2.591	**8**	_	1.0000	0.9943	0.9504	0.9116	0.8629	0.6333	0.6533	0.3362	0.2070	0.0808	0.1109	0.0971	0.0423	0.0139	0.0039	0.0040	0.0016	0.0164	<.0001	<.0001
Pilcraw	40.6922	2.399	**10**	1.0000	_	1.0000	0.9999	0.9996	0.9987	0.9758	0.9763	0.8194	0.6766	0.3919	0.4628	0.4114	0.2503	0.1060	0.0367	0.0347	0.0161	0.0966	0.0007	0.0003
Arco	37.7333	2.770	**2**	0.9943	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9996	0.9976	0.9682	0.9764	0.9578	0.9102	0.7322	0.4815	0.4354	0.3199	0.5763	0.0464	0.0261
Canus	36.9289	2.399	**4**	0.9504	0.9999	1.0000	_	1.0000	1.0000	1.0000	1.0000	0.9999	0.9993	0.9819	0.9876	0.9748	0.9360	0.7654	0.4985	0.4533	0.3224	0.6164	0.0389	0.0206
Spinkcota	36.4822	2.399	**14**	0.9116	0.9996	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	0.9998	0.9928	0.9952	0.9885	0.9672	0.8425	0.5970	0.5469	0.4093	0.6969	0.0577	0.0314
Bunyip	36.0800	2.399	**3**	0.8629	0.9987	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	0.9973	0.9982	0.9950	0.9840	0.8977	0.6841	0.6321	0.4948	0.7642	0.0808	0.0453
White Marquis	34.8036	2.399	**20**	0.6333	0.9758	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9993	0.9857	0.9003	0.8621	0.7664	0.9216	0.2086	0.1290
Onas	34.5739	2.591	**7**	0.6533	0.9763	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9998	0.9944	0.9475	0.9201	0.8549	0.9534	0.3094	0.2060
Surprise	32.9589	2.591	**15**	0.3362	0.8194	0.9996	0.9999	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9977	0.9942	0.9844	0.9965	0.6338	0.4899
Sonora	32.5178	2.399	**13**	0.2070	0.6766	0.9976	0.9993	0.9998	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9988	0.9967	0.9898	0.9981	0.6552	0.5057
Penawawa	31.2000	2.399	**9**	0.0808	0.3919	0.9682	0.9819	0.9928	0.9973	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9997	1.0000	0.8888	0.7833
Zak	31.1661	2.591	**21**	0.1109	0.4628	0.9764	0.9876	0.9952	0.9982	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	1.0000	0.9232	0.8397
Wawawai	30.5428	2.770	**17**	0.0971	0.4114	0.9578	0.9748	0.9885	0.9950	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9781	0.9405
Red Fife	30.4144	2.399	**11**	0.0423	0.2503	0.9102	0.9360	0.9672	0.9840	0.9993	0.9998	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9613	0.9017
Wakanz	29.1956	2.399	**16**	0.0139	0.1060	0.7322	0.7654	0.8425	0.8977	0.9857	0.9944	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	0.9967	0.9850
White Federation	27.9411	2.399	**19**	0.0039	0.0367	0.4815	0.4985	0.5970	0.6841	0.9003	0.9475	0.9977	0.9988	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	0.9999	0.9994
Currawa	27.3567	2.591	**5**	0.0040	0.0347	0.4354	0.4533	0.5469	0.6321	0.8621	0.9201	0.9942	0.9967	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000
Idaed	27.0756	2.399	**6**	0.0016	0.0161	0.3199	0.3224	0.4093	0.4948	0.7664	0.8549	0.9844	0.9898	0.9997	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000
Alpowa	26.8678	3.249	**1**	0.0164	0.0966	0.5763	0.6164	0.6969	0.7642	0.9216	0.9534	0.9965	0.9981	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000
Westbred Express	24.2433	2.399	**18**	<.0001	0.0007	0.0464	0.0389	0.0577	0.0808	0.2086	0.3094	0.6338	0.6552	0.8888	0.9232	0.9781	0.9613	0.9967	0.9999	1.0000	1.0000	1.0000	_	1.0000
Scarlet	23.5633	2.399	**12**	<.0001	0.0003	0.0261	0.0206	0.0314	0.0453	0.1290	0.2060	0.4899	0.5057	0.7833	0.8397	0.9405	0.9017	0.9850	0.9994	1.0000	1.0000	1.0000	1.0000	_

Table B.6: Genotype LS mean values and pairwise comparisons for grain total N (TN) and straw weight for annual genotypes at maturity. Significant differences at the p<0.05 level are shaded.

genotype	straw %N	std err*	entry	_16	_1	_18	_15	_12	_21	_5	_19	_6	_9	_8	_10	_17	_20	_2	_13	_14	_7	_3	_11	_4
Wakanz	0.5011	1.080	**16**	_	1.0000	1.0000	0.9659	0.9097	0.7492	0.2184	0.1378	0.0933	0.0411	0.0502	0.0148	0.0207	0.0056	0.0133	0.0042	0.0018	0.0024	0.0009	0.0007	<.0001
Alpowa	0.4993	1.110	**1**	1.0000	_	1.0000	0.9954	0.9857	0.9323	0.5339	0.4361	0.3504	0.2150	0.2245	0.1126	0.1159	0.0592	0.0857	0.0484	0.0271	0.0286	0.0173	0.0141	0.0013
Westbred Express	0.4950	1.080	**18**	1.0000	1.0000	_	0.9819	0.9452	0.8152	0.2745	0.1805	0.1251	0.0573	0.0686	0.0215	0.0289	0.0084	0.0188	0.0063	0.0027	0.0036	0.0015	0.0011	<.0001
Surprise	0.4090	1.087	**15**	0.9659	0.9954	0.9819	_	1.0000	1.0000	0.9996	0.9987	0.9952	0.9689	0.9667	0.8741	0.8505	0.7182	0.7779	0.6631	0.5032	0.4975	0.3905	0.3447	0.0550
Scarlet	0.4033	1.080	**12**	0.9097	0.9857	0.9452	1.0000	_	1.0000	0.9998	0.9994	0.9973	0.9777	0.9760	0.8936	0.8725	0.7409	0.8026	0.6849	0.5193	0.5156	0.4009	0.3527	0.0521
Zak	0.3861	1.087	**21**	0.7492	0.9323	0.8152	1.0000	1.0000	_	1.0000	1.0000	1.0000	0.9995	0.9993	0.9917	0.9851	0.9564	0.9676	0.9361	0.8495	0.8358	0.7577	0.7113	0.2075
Currawa	0.3532	1.087	**5**	0.2184	0.5339	0.2745	0.9996	0.9998	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9989	0.9981	0.9952	0.9919	0.7340
White Federation	0.3506	1.080	**19**	0.1378	0.4361	0.1805	0.9987	0.9994	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9990	0.9983	0.9955	0.9921	0.7159
Idaed	0.3450	1.080	**6**	0.0933	0.3504	0.1251	0.9952	0.9973	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9998	0.9996	0.9988	0.9977	0.8112
Penawawa	0.3345	1.080	**9**	0.0411	0.2150	0.0573	0.9689	0.9777	0.9995	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9360
Pacific Bluestem	0.3315	1.087	**8**	0.0502	0.2245	0.0686	0.9667	0.9760	0.9993	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9713
Pilcraw	0.3231	1.080	**10**	0.0148	0.1126	0.0215	0.8741	0.8936	0.9917	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9899
Wawawai	0.3157	1.093	**17**	0.0207	0.1159	0.0289	0.8505	0.8725	0.9851	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9994
White Marquis	0.3136	1.080	**20**	0.0056	0.0592	0.0084	0.7182	0.7409	0.9564	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9991
Arco	0.3108	1.093	**2**	0.0133	0.0857	0.0188	0.7779	0.8026	0.9676	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999
Sonora	0.3108	1.080	**13**	0.0042	0.0484	0.0063	0.6631	0.6849	0.9361	0.9999	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	0.9996
Spinkcota	0.3034	1.080	**14**	0.0018	0.0271	0.0027	0.5032	0.5193	0.8495	0.9989	0.9990	0.9998	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000
Onas	0.2997	1.087	**7**	0.0024	0.0286	0.0036	0.4975	0.5156	0.8358	0.9981	0.9983	0.9996	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000
Bunyip	0.2980	1.080	**3**	0.0009	0.0173	0.0015	0.3905	0.4009	0.7577	0.9952	0.9955	0.9988	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000
Red Fife	0.2957	1.080	**11**	0.0007	0.0141	0.0011	0.3447	0.3527	0.7113	0.9919	0.9921	0.9977	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000
Canus	0.2715	1.080	**4**	<.0001	0.0013	<.0001	0.0550	0.0521	0.2075	0.7340	0.7159	0.8112	0.9360	0.9713	0.9899	0.9994	0.9991	0.9999	0.9996	1.0000	1.0000	1.0000	1.0000	_

genotype	straw TN (g)	std err	entry	_16	_8	_15	_10	_1	_21	_18	_2	_14	_9	_20	_3	_13	_7	_4	_19	_5	_12	_17	_11	_6
Wakanz	0.1528	0.00964	**16**	_	1.0000	0.9992	0.9967	0.9990	0.9146	0.7580	0.6890	0.4085	0.3540	0.2401	0.1466	0.1254	0.1683	0.0567	0.0402	0.0516	0.0119	0.0317	0.0101	0.0095
Pacific Bluestem	0.1450	0.01041	**8**	1.0000	_	1.0000	1.0000	1.0000	0.9984	0.9860	0.9655	0.8597	0.8187	0.6991	0.5482	0.5035	0.5672	0.3124	0.2495	0.2764	0.1055	0.1860	0.0934	0.0894
Surprise	0.1342	0.01041	**15**	0.9992	1.0000	_	1.0000	1.0000	1.0000	1.0000	0.9999	0.9987	0.9974	0.9884	0.9597	0.9455	0.9592	0.8336	0.7684	0.7820	0.5094	0.6407	0.4758	0.4639
Pilcraw	0.1329	0.00964	**10**	0.9967	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	0.9992	0.9982	0.9909	0.9648	0.9513	0.9647	0.8397	0.7728	0.7887	0.5037	0.6452	0.4687	0.4564
Alpowa	0.1311	0.01305	**1**	0.9990	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9997	0.9980	0.9968	0.9976	0.9807	0.9660	0.9654	0.8618	0.9088	0.8417	0.8341
Zak	0.1246	0.01041	**21**	0.9146	0.9984	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9998	0.9998	0.9959	0.9903	0.9901	0.9245	0.9594	0.9083	0.9020
Westbred Express	0.1216	0.00964	**18**	0.7580	0.9860	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9991	0.9973	0.9971	0.9627	0.9824	0.9523	0.9481
Arco	0.1176	0.01113	**2**	0.6890	0.9655	0.9999	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9980	0.9991	0.9970	0.9965
Spinkcota	0.1150	0.00964	**14**	0.4085	0.8597	0.9987	0.9992	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9990	0.9997	0.9985	0.9982
Penawawa	0.1139	0.00964	**9**	0.3540	0.8187	0.9974	0.9982	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9996	0.9999	0.9993	0.9992
White Marquis	0.1112	0.00964	**20**	0.2401	0.6991	0.9884	0.9909	0.9997	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9999
Bunyip	0.1083	0.00964	**3**	0.1466	0.5482	0.9597	0.9648	0.9980	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
Sonora	0.1075	0.00964	**13**	0.1254	0.5035	0.9455	0.9513	0.9968	0.9998	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
Onas	0.1073	0.01041	**7**	0.1683	0.5672	0.9592	0.9647	0.9976	0.9998	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
Canus	0.1035	0.00964	**4**	0.0567	0.3124	0.8336	0.8397	0.9807	0.9959	0.9991	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
White Federation	0.1020	0.00964	**19**	0.0402	0.2495	0.7684	0.7728	0.9660	0.9903	0.9973	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000
Currawa	0.1011	0.01041	**5**	0.0516	0.2764	0.7820	0.7887	0.9654	0.9901	0.9971	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000
Scarlet	0.0969	0.00964	**12**	0.0119	0.1055	0.5094	0.5037	0.8618	0.9245	0.9627	0.9980	0.9990	0.9996	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000
Wawawai	0.0968	0.01113	**17**	0.0317	0.1860	0.6407	0.6452	0.9088	0.9594	0.9824	0.9991	0.9997	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000
Red Fife	0.0962	0.00964	**11**	0.0101	0.0934	0.4758	0.4687	0.8417	0.9083	0.9523	0.9970	0.9985	0.9993	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000
Idaed	0.0960	0.00964	**6**	0.0095	0.0894	0.4639	0.4564	0.8341	0.9020	0.9481	0.9965	0.9982	0.9992	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_

Table B.7: Genotype LS mean values and pairwise comparisons for straw N concentration (%N) and straw total N (TN) for annual genotypes at maturity. Significant differences at the p<0.05 level are shaded. * Straw %N was analysed using a logarithmic transformation of the data. Therefore, the standard error is a percentage, rather than an absolute estimation of the error. To get one standard deviation on either side of the point estimate, multiply and divide the point estimate by the standard error, rather than adding and subtracting the standard error as would be done for the untransformed variables.

genotype	biomass (g)	std err	entry	_10	_3	_7	_4	_8	_17	_2	_14	_9	_20	_21	_15	_19	_13	_16	_6	_11	_5	_1	_12	_18
Pilcraw	63.27	3.259	**10**	_	1.0000	1.0000	0.9999	0.9998	0.9997	0.9994	0.9894	0.9870	0.9852	0.9594	0.7183	0.3367	0.1849	0.1412	0.0674	0.0613	0.0814	0.1972	0.0010	0.0007
Bunyip	60.04	3.259	**3**	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9831	0.8242	0.6359	0.5523	0.3546	0.3337	0.3812	0.5732	0.0134	0.0103
Onas	59.17	3.520	**7**	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9972	0.9377	0.8212	0.7558	0.5651	0.5416	0.5830	0.7367	0.0405	0.0323
Canus	58.05	3.259	**4**	0.9999	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9995	0.9740	0.8966	0.8445	0.6687	0.6449	0.6852	0.8221	0.0533	0.0425
Pacific Bluestem	57.56	3.520	**8**	0.9998	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9915	0.9542	0.9238	0.7994	0.7803	0.8063	0.8942	0.1055	0.0867
Wawawai	57.21	3.763	**17**	0.9997	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9969	0.9788	0.9616	0.8787	0.8646	0.8802	0.9349	0.1714	0.1446
Arco	56.84	3.763	**2**	0.9994	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9985	0.9870	0.9749	0.9098	0.8981	0.9101	0.9524	0.2043	0.1736
Spinkcota	55.85	3.259	**14**	0.9894	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	0.9995	0.9929	0.9842	0.9286	0.9177	0.9300	0.9678	0.1881	0.1572
Penawawa	55.72	3.259	**9**	0.9870	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	0.9996	0.9943	0.9870	0.9374	0.9274	0.9383	0.9720	0.2010	0.1684
White Marquis	55.63	3.259	**20**	0.9852	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	0.9997	0.9951	0.9886	0.9428	0.9334	0.9434	0.9745	0.2098	0.1762
Zak	54.50	3.520	**21**	0.9594	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	0.9997	0.9991	0.9910	0.9887	0.9902	0.9955	0.4234	0.3733
Surprise	52.00	3.520	**15**	0.7183	0.9831	0.9972	0.9995	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	0.9999	0.9999	1.0000	0.7959	0.7501
White Federation	49.99	3.259	**19**	0.3367	0.8242	0.9377	0.9740	0.9915	0.9969	0.9985	0.9995	0.9996	0.9997	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9455	0.9245
Sonora	48.68	3.259	**13**	0.1849	0.6359	0.8212	0.8966	0.9542	0.9788	0.9870	0.9929	0.9943	0.9951	0.9997	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9899	0.9831
Wakanz	48.17	3.259	**16**	0.1412	0.5523	0.7558	0.8445	0.9238	0.9616	0.9749	0.9842	0.9870	0.9886	0.9991	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	0.9957	0.9923
Idaed	46.89	3.259	**6**	0.0674	0.3546	0.5651	0.6687	0.7994	0.8787	0.9098	0.9286	0.9374	0.9428	0.9910	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	0.9997	0.9994
Red Fife	46.74	3.259	**11**	0.0613	0.3337	0.5416	0.6449	0.7803	0.8646	0.8981	0.9177	0.9274	0.9334	0.9887	0.9999	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	0.9998	0.9996
Currawa	46.55	3.520	**5**	0.0814	0.3812	0.5830	0.6852	0.8063	0.8802	0.9101	0.9300	0.9383	0.9434	0.9902	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	0.9999	0.9999
Alpowa	46.06	4.412	**1**	0.1972	0.5732	0.7367	0.8221	0.8942	0.9349	0.9524	0.9678	0.9720	0.9745	0.9955	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000
Scarlet	41.30	3.259	**12**	0.0010	0.0134	0.0405	0.0533	0.1055	0.1714	0.2043	0.1881	0.2010	0.2098	0.4234	0.7959	0.9455	0.9899	0.9957	0.9997	0.9998	0.9999	1.0000	_	1.0000
Westbred Express	40.95	3.259	**18**	0.0007	0.0103	0.0323	0.0425	0.0867	0.1446	0.1736	0.1572	0.1684	0.1762	0.3733	0.7501	0.9228	0.9831	0.9923	0.9994	0.9996	0.9999	1.0000	1.0000	_

genotype	HI	std err	entry	_17	_19	_9	_1	_12	_21	_6	_18	_5	_7	_3	_16	_20	_4	_10	_15	_14	_11	_2	_13	_8
Wawawai	0.4683	0.02766	**17**	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9996	0.9978	0.9958	0.9815	0.9214	0.7553	0.5964	0.3591	0.3420	0.3737	0.1377	0.1254	0.1623	0.0081	<.0001
White Federation	0.4429	0.02395	**19**	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9985	0.9766	0.9195	0.7348	0.7153	0.7423	0.3893	0.3625	0.4258	0.0321	<.0001
Penawawa	0.4345	0.02395	**9**	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9961	0.9777	0.8771	0.8637	0.8777	0.5705	0.5406	0.5960	0.0676	<.0001
Alpowa	0.4340	0.03243	**1**	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9997	0.9974	0.9763	0.9727	0.9734	0.8506	0.8329	0.8412	0.2821	0.0011
Scarlet	0.4330	0.02395	**12**	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9973	0.9830	0.8961	0.8839	0.8959	0.6035	0.5735	0.6263	0.0765	<.0001
Zak	0.4272	0.02587	**21**	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9997	0.9970	0.9671	0.9618	0.9648	0.7862	0.7620	0.7893	0.1677	0.0002
Idaed	0.4228	0.02395	**6**	0.9996	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	0.9999	0.9984	0.9762	0.9719	0.9745	0.8088	0.7849	0.8137	0.1671	0.0001
Westbred Express	0.4165	0.02395	**18**	0.9978	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	0.9998	0.9933	0.9917	0.9923	0.9005	0.8838	0.8983	0.2543	0.0003
Currawa	0.4119	0.02587	**5**	0.9958	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	0.9988	0.9984	0.9984	0.9626	0.9543	0.9574	0.4093	0.0010
Onas	0.4042	0.02587	**7**	0.9815	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	0.9999	0.9999	0.9998	0.9905	0.9876	0.9874	0.5696	0.0023
Bunyip	0.3964	0.02395	**3**	0.9214	0.9985	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9975	0.9965	0.9962	0.6640	0.0027
Wakanz	0.3843	0.02395	**16**	0.7553	0.9766	0.9961	0.9997	0.9973	0.9997	0.9999	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9999	0.8798	0.0095
White Marquis	0.3762	0.02395	**20**	0.5964	0.9195	0.9777	0.9974	0.9830	0.9970	0.9984	0.9998	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9590	0.0208
Canus	0.3641	0.02395	**4**	0.3591	0.7348	0.8771	0.9763	0.8961	0.9671	0.9762	0.9933	0.9988	0.9999	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9962	0.0598
Pilcraw	0.3632	0.02395	**10**	0.3420	0.7153	0.8637	0.9727	0.8839	0.9618	0.9719	0.9917	0.9984	0.9999	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	0.9971	0.0647
Surprise	0.3613	0.02587	**15**	0.3737	0.7423	0.8777	0.9734	0.8959	0.9648	0.9745	0.9923	0.9984	0.9998	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	0.9990	0.1064
Spinkcota	0.3480	0.02395	**14**	0.1377	0.3893	0.5705	0.8506	0.6035	0.7862	0.8088	0.9005	0.9626	0.9905	0.9975	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	0.1969
Red Fife	0.3467	0.02395	**11**	0.1254	0.3625	0.5406	0.8329	0.5735	0.7620	0.7849	0.8838	0.9543	0.9876	0.9965	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	0.2147
Arco	0.3423	0.02766	**2**	0.1623	0.4258	0.5960	0.8412	0.6263	0.7893	0.8137	0.8983	0.9574	0.9874	0.9962	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	0.4103
Sonora	0.3142	0.02395	**13**	0.0081	0.0321	0.0676	0.2821	0.0765	0.1677	0.1671	0.2543	0.4093	0.5696	0.6640	0.8798	0.9590	0.9962	0.9971	0.9990	1.0000	1.0000	1.0000	_	0.8263
Pacific Bluestem	0.2374	0.02587	**8**	<.0001	<.0001	<.0001	0.0011	<.0001	0.0002	0.0001	0.0003	0.0010	0.0023	0.0027	0.0095	0.0208	0.0598	0.0647	0.1064	0.1969	0.2147	0.4103	0.8263	_

Table B.8: Genotype LS mean values and pairwise comparisons for aboveground biomass and harvest index (HI) at maturity. Significant differences at the p<0.05 level are shaded.

genotype	NHI	std err	entry	_19	_17	_5	_6	_4	_20	_7	_3	_14	_9	_12	_11	_18	_21	_13	_2	_1	_10	_15	_8	_16
White Federation	0.8146	0.02798	**19**	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9999	0.9999	0.9989	0.9974	0.8941	0.9439	0.9405	0.3902	0.1530	0.0401	0.0153
Wawawai	0.8061	0.03231	**17**	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9820	0.9912	0.9877	0.6882	0.3718	0.1406	0.0719
Currawa	0.8051	0.03023	**5**	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9771	0.9891	0.9855	0.6403	0.3228	0.1112	0.0524
Idaed	0.8037	0.02798	**6**	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9998	0.9718	0.9872	0.9837	0.5922	0.2781	0.0867	0.0373
Canus	0.7877	0.02798	**4**	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9987	0.9995	0.9990	0.8571	0.5427	0.2270	0.1179
White Marquis	0.7845	0.02798	**20**	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9995	0.9998	0.9995	0.8938	0.6006	0.2676	0.1445
Onas	0.7838	0.03023	**7**	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9998	0.9999	0.9997	0.9297	0.6806	0.3435	0.2045
Bunyip	0.7826	0.02798	**3**	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9997	0.9999	0.9997	0.9130	0.6358	0.2947	0.1630
Spinkcota	0.7784	0.02798	**14**	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	1.0000	0.9999	0.9457	0.7078	0.3569	0.2078
Penawawa	0.7701	0.02798	**9**	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9834	0.8340	0.4990	0.3223
Scarlet	0.7697	0.02798	**12**	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9846	0.8401	0.5075	0.3297
Red Fife	0.7686	0.02798	**11**	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9871	0.8531	0.5263	0.3465
Westbred Express	0.7615	0.02798	**18**	0.9989	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	0.9967	0.9249	0.6544	0.4701
Zak	0.7556	0.03023	**21**	0.9974	0.9999	0.9999	0.9998	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	0.9996	0.9745	0.8069	0.6564
Sonora	0.7340	0.02798	**13**	0.8941	0.9820	0.9771	0.9718	0.9987	0.9995	0.9998	0.9997	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	0.9994	0.9703	0.9124
Arco	0.7338	0.03231	**2**	0.9439	0.9912	0.9891	0.9872	0.9995	0.9998	0.9999	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	0.9998	0.9865	0.9571
Alpowa	0.7249	0.03789	**1**	0.9405	0.9877	0.9855	0.9837	0.9990	0.9995	0.9997	0.9997	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	0.9991	0.9961
Pilcraw	0.7038	0.02798	**10**	0.3902	0.6882	0.6403	0.5922	0.8571	0.8938	0.9297	0.9130	0.9457	0.9834	0.9846	0.9871	0.9967	0.9996	1.0000	1.0000	1.0000	_	1.0000	1.0000	0.9997
Surprise	0.6810	0.03023	**15**	0.1530	0.3718	0.3228	0.2781	0.5427	0.6006	0.6806	0.6358	0.7078	0.8340	0.8401	0.8531	0.9249	0.9745	0.9994	0.9998	1.0000	1.0000	_	1.0000	1.0000
Pacific Bluestem	0.6610	0.03023	**8**	0.0401	0.1406	0.1112	0.0867	0.2270	0.2676	0.3435	0.2947	0.3569	0.4990	0.5075	0.5263	0.6544	0.8069	0.9703	0.9865	0.9991	1.0000	1.0000	_	1.0000
Wakanz	0.6552	0.02798	**16**	0.0153	0.0719	0.0524	0.0373	0.1179	0.1445	0.2045	0.1630	0.2078	0.3223	0.3297	0.3465	0.4701	0.6564	0.9124	0.9571	0.9961	0.9997	1.0000	1.0000	_

Table B.9: Genotype LS mean values and pairwise comparisons for nitrogen harvest index (NHI) and total N uptake (N up) at maturity. Significant differences at the $p<0.05$ level are shaded.

genotype	N up (g)	std err	entry	_19	_20	_6	_21	_5	_14	_7	_3	_17	_15	_4	_18	_9	_10	_16	_1	_2	_12	_11	_13	_8
White Federation	0.5513	0.02359	**19**	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9998	0.9996	0.9993	0.9952	0.9937	0.9814	0.9430	0.9337	0.7265	0.4556	0.4205	0.1905	0.0892
White Marquis	0.5467	0.02359	**20**	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9998	0.9986	0.9980	0.9927	0.9721	0.9623	0.8062	0.5559	0.5191	0.2569	0.1257
Idaed	0.5318	0.02359	**6**	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9992	0.9973	0.9656	0.8551	0.8293	0.5593	0.3304
Zak	0.5269	0.02548	**21**	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9994	0.9896	0.9415	0.9273	0.7335	0.4962
Currawa	0.5229	0.02548	**5**	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9998	0.9954	0.9678	0.9585	0.8065	0.5795
Spinkcota	0.5198	0.02359	**14**	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9965	0.9713	0.9623	0.8081	0.5745
Onas	0.5158	0.02548	**7**	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9992	0.9915	0.9880	0.9067	0.7236
Bunyip	0.5153	0.02359	**3**	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9989	0.9877	0.9829	0.8780	0.6704
Wawawai	0.5083	0.02724	**17**	0.9998	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9992	0.9987	0.9772	0.8835
Surprise	0.5082	0.02548	**15**	0.9996	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9987	0.9979	0.9672	0.8505
Canus	0.5078	0.02359	**4**	0.9993	0.9998	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9980	0.9969	0.9549	0.8137
Westbred Express	0.5011	0.02359	**18**	0.9952	0.9986	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9998	0.9996	0.9862	0.9063
Penawawa	0.4999	0.02359	**9**	0.9937	0.9980	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	0.9998	0.9997	0.9890	0.9182
Pilcraw	0.4948	0.02359	**10**	0.9814	0.9927	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9967	0.9598
Wakanz	0.4880	0.02359	**16**	0.9430	0.9721	0.9992	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	0.9996	0.9876
Alpowa	0.4747	0.03194	**1**	0.9337	0.9623	0.9973	0.9994	0.9998	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000
Arco	0.4670	0.02724	**2**	0.7265	0.8062	0.9656	0.9896	0.9954	0.9965	0.9992	0.9989	1.0000	0.9999	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000
Scarlet	0.4609	0.02359	**12**	0.4556	0.5559	0.8551	0.9415	0.9678	0.9713	0.9915	0.9877	0.9992	0.9987	0.9980	0.9998	0.9998	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000
Red Fife	0.4593	0.02359	**11**	0.4205	0.5191	0.8293	0.9273	0.9585	0.9623	0.9880	0.9829	0.9987	0.9979	0.9969	0.9996	0.9997	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000
Sonora	0.4461	0.02359	**13**	0.1905	0.2569	0.5593	0.7335	0.8065	0.8081	0.9067	0.8780	0.9772	0.9672	0.9549	0.9862	0.9890	0.9967	0.9996	1.0000	1.0000	1.0000	1.0000	_	1.0000
Pacific Bluestem	0.4314	0.02548	**8**	0.0892	0.1257	0.3304	0.4962	0.5795	0.5745	0.7236	0.6704	0.8835	0.8505	0.8137	0.9063	0.9182	0.9598	0.9876	1.0000	1.0000	1.0000	1.0000	1.0000	_

genotype	N ut	std err	entry	_17	_9	_7	_3	_21	_10	_4	_2	_19	_12	_1	_6	_20	_11	_14	_5	_15	_16	_13	_18	_8
Wawawai	54.48	3.450	**17**	_	1.0000	0.9999	0.9980	0.9946	0.9915	0.8841	0.8534	0.6112	0.5001	0.7628	0.2594	0.2265	0.1152	0.1120	0.1462	0.1457	0.0505	0.0392	0.0101	0.0037
Penawawa	52.38	2.988	**9**	1.0000	_	1.0000	1.0000	0.9999	0.9997	0.9764	0.9621	0.8116	0.7088	0.9098	0.4192	0.3723	0.2002	0.1949	0.2503	0.2495	0.0898	0.0699	0.0176	0.0064
Onas	48.98	3.228	**7**	0.9999	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9989	0.9955	0.9994	0.9482	0.9295	0.7941	0.7872	0.8291	0.8283	0.5798	0.5149	0.2364	0.1089
Bunyip	48.05	2.988	**3**	0.9980	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	0.9998	0.9989	0.9999	0.9761	0.9646	0.8634	0.8577	0.8912	0.8906	0.6647	0.5981	0.2875	0.1346
Zak	47.30	3.228	**21**	0.9946	0.9999	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	0.9999	1.0000	0.9956	0.9926	0.9540	0.9513	0.9640	0.9637	0.8363	0.7864	0.4795	0.2601
Pilcraw	47.27	2.988	**10**	0.9915	0.9997	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	0.9999	1.0000	0.9932	0.9888	0.9357	0.9322	0.9504	0.9501	0.7897	0.7315	0.4062	0.2060
Canus	45.07	2.988	**4**	0.8841	0.9764	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9981	0.9979	0.9986	0.9986	0.9781	0.9633	0.7805	0.5224
Arco	44.10	3.450	**2**	0.8534	0.9621	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	1.0000	1.0000	0.9984	0.9966	0.9481	0.7952
White Federation	43.07	2.988	**19**	0.6112	0.8116	0.9989	0.9998	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9996	0.9990	0.9681	0.8341
Scarlet	42.40	2.988	**12**	0.5001	0.7088	0.9955	0.9989	0.9999	0.9999	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9998	0.9883	0.9055
Alpowa	42.33	4.046	**1**	0.7628	0.9098	0.9994	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9988	0.9811
Idaed	40.73	2.988	**6**	0.2594	0.4192	0.9482	0.9761	0.9956	0.9932	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9997	0.9877
White Marquis	40.44	2.988	**20**	0.2265	0.3723	0.9295	0.9646	0.9926	0.9888	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9999	0.9923
Red Fife	39.15	2.988	**11**	0.1152	0.2002	0.7941	0.8634	0.9540	0.9357	0.9981	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9995
Spinkcota	39.11	2.988	**14**	0.1120	0.1949	0.7872	0.8577	0.9513	0.9322	0.9979	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	1.0000	0.9995
Currawa	39.06	3.228	**5**	0.1462	0.2503	0.8291	0.8912	0.9640	0.9504	0.9986	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	1.0000	0.9998
Surprise	39.05	3.228	**15**	0.1457	0.2495	0.8283	0.8906	0.9637	0.9501	0.9986	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000	0.9998
Wakanz	37.80	2.988	**16**	0.0505	0.0898	0.5798	0.6647	0.8363	0.7897	0.9781	0.9984	0.9996	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000	1.0000
Sonora	37.42	2.988	**13**	0.0392	0.0699	0.5149	0.5981	0.7864	0.7315	0.9633	0.9966	0.9990	0.9998	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000	1.0000
Westbred Express	35.54	2.988	**18**	0.0101	0.0176	0.2364	0.2875	0.4795	0.4062	0.7805	0.9481	0.9681	0.9883	0.9988	0.9997	0.9999	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	_	1.0000
Pacific Bluestem	33.56	3.228	**8**	0.0037	0.0064	0.1089	0.1346	0.2601	0.2060	0.5224	0.7952	0.8341	0.9055	0.9811	0.9877	0.9923	0.9995	0.9995	0.9998	0.9998	1.0000	1.0000	1.0000	_

Table B.10: Genotype LS mean values and pairwise comparisons for N utilization efficiency at maturity. Significant differences at the p<0.05 level are shaded.

genotype	weight (g)	std err	entry	_1	_6	_2	_8	_7	_5	_3	_9	_10	_4
AgCs	2.00	0.185	**1**	-	0.0976	0.0202	0.0133	0.0086	0.0032	0.0037	0.0015	0.0006	0.0004
Cs+4E	1.21	0.185	**6**	0.0976	-	0.9998	0.9990	0.9962	0.9671	0.9546	0.9111	0.7807	0.4005
Cs	1.05	0.185	**2**	0.0202	0.9998	-	1.0000	1.0000	0.9998	0.9994	0.9977	0.9810	0.7314
Cs+6E	1.01	0.185	**8**	0.0133	0.9990	1.0000	-	1.0000	1.0000	0.9999	0.9995	0.9928	0.8014
Cs+5E	0.98	0.185	**7**	0.0086	0.9962	1.0000	1.0000	-	1.0000	1.0000	0.9999	0.9978	0.8614
Cs+3E	0.89	0.185	**5**	0.0032	0.9671	0.9998	1.0000	1.0000	-	1.0000	1.0000	1.0000	0.9522
Cs+1E	0.86	0.200	**3**	0.0037	0.9546	0.9994	0.9999	1.0000	1.0000	-	1.0000	1.0000	0.9758
Cs+7E	0.83	0.185	**9**	0.0015	0.9111	0.9977	0.9995	0.9999	1.0000	1.0000	-	1.0000	0.9824
Elongatum	0.76	0.185	**10**	0.0006	0.7807	0.9810	0.9928	0.9978	1.0000	1.0000	1.0000	-	0.9970
Cs+2E	0.49	0.251	**4**	0.0004	0.4005	0.7314	0.8014	0.8614	0.9522	0.9758	0.9824	0.9970	-

genotype	%N	std err	entry	_9	_4	_3	_8	_7	_6	_5	_2	_10	_1
Cs+7E	6.143	0.117	**9**	-	1.0000	0.9915	0.7253	0.8261	0.1156	0.0169	0.0046	<.0001	0.0634
Cs+2E	6.064	0.180	**4**	1.0000	-	1.0000	0.9966	0.9965	0.7883	0.3770	0.1871	0.0086	0.2060
Cs+1E	5.996	0.097	**3**	0.9915	1.0000	-	0.9976	0.9984	0.4633	0.0780	0.0199	<.0001	0.1568
Cs+6E	5.899	0.068	**8**	0.7253	0.9966	0.9976	-	1.0000	0.7650	0.1345	0.0290	<.0001	0.2538
Cs+5E	5.882	0.111	**7**	0.8261	0.9965	0.9984	1.0000	-	0.9797	0.5408	0.2245	0.0016	0.3488
Cs+4E	5.742	0.060	**6**	0.1156	0.7883	0.4633	0.7650	0.9797	-	0.8892	0.4252	0.0003	0.5764
Cs+3E	5.601	0.074	**5**	0.0169	0.3770	0.0780	0.1345	0.5408	0.8892	-	0.9975	0.0444	0.8845
Cs	5.511	0.081	**2**	0.0046	0.1871	0.0199	0.0290	0.2245	0.4252	0.9975	-	0.3485	0.9748
Elongatum	5.265	0.060	**10**	<.0001	0.0086	<.0001	<.0001	0.0016	0.0003	0.0444	0.3485	-	1.0000
AgCs	5.204	0.256	**1**	0.0634	0.2060	0.1568	0.2538	0.3488	0.5764	0.8845	0.9748	1.0000	-

genotype	TN (g)	std err	entry	_1	_6	_2	_8	_7	_5	_9	_3	_10	_4
AgCs	0.1022	0.00941	**1**	-	0.3095	0.0586	0.0341	0.0313	0.0127	0.0055	0.0034	0.0003	0.0006
Cs+4E	0.0694	0.00941	**6**	0.3095	-	0.9988	0.9926	0.9908	0.9442	0.8437	0.7051	0.2874	0.2111
Cs	0.0592	0.00941	**2**	0.0586	0.9988	-	1.0000	1.0000	0.9999	0.9978	0.9821	0.7687	0.5858
Cs+6E	0.0564	0.00941	**8**	0.0341	0.9926	1.0000	-	1.0000	1.0000	0.9998	0.9956	0.8728	0.6998
Cs+5E	0.0560	0.00941	**7**	0.0313	0.9908	1.0000	1.0000	-	1.0000	0.9998	0.9966	0.8855	0.7160
Cs+3E	0.0518	0.00941	**5**	0.0127	0.9442	0.9999	1.0000	1.0000	-	1.0000	0.9999	0.9717	0.8612
Cs+7E	0.0481	0.00941	**9**	0.0055	0.8437	0.9978	0.9998	0.9998	1.0000	-	1.0000	0.9955	0.9427
Cs+1E	0.0439	0.01016	**3**	0.0034	0.7051	0.9821	0.9956	0.9966	0.9999	1.0000	-	0.9999	0.9892
Elongatum	0.0360	0.00941	**10**	0.0003	0.2874	0.7687	0.8728	0.8855	0.9717	0.9955	0.9999	-	0.9999
Cs+2E	0.0271	0.01274	**4**	0.0006	0.2111	0.5858	0.6998	0.7160	0.8612	0.9427	0.9892	0.9999	-

Table B.11: Genotype LS mean values and pairwise comparisons for aboveground biomass (weight) and tissue N concentration (%N) and total plant N (TN) for addition genotypes at the 6leaf stage. Significant differences at the $p<0.05$ level are shaded.

genotype	weight (g)	std err	entry	_3	_2	_9	_1	_5	_8	_6	_7	_10	_4
Cs+1E	38.81	2.255	**3**	-	1.0000	0.9996	0.9758	0.8797	0.8232	0.9071	0.7279	0.3445	0.0170
Cs	37.91	2.348	**2**	1.0000	-	1.0000	0.9961	0.9592	0.9415	0.9774	0.8755	0.5503	0.0474
Cs+7E	36.75	2.164	**9**	0.9996	1.0000	-	0.9995	0.9978	0.9937	0.9956	0.9823	0.6196	0.0518
AgCs	34.21	2.835	**1**	0.9758	0.9961	0.9995	-	1.0000	1.0000	1.0000	1.0000	0.8863	0.1816
Cs+3E	34.03	2.675	**5**	0.8797	0.9592	0.9978	1.0000	-	1.0000	1.0000	1.0000	0.9917	0.5523
Cs+6E	33.85	2.227	**8**	0.8232	0.9415	0.9937	1.0000	1.0000	-	1.0000	1.0000	0.9809	0.3726
Cs+4E	33.71	2.404	**6**	0.9071	0.9774	0.9956	1.0000	1.0000	1.0000	-	1.0000	0.9439	0.2168
Cs+5E	33.28	2.446	**7**	0.7279	0.8755	0.9823	1.0000	1.0000	1.0000	1.0000	-	0.9965	0.5875
Elongatum	29.65	2.675	**10**	0.3445	0.5503	0.6196	0.8863	0.9917	0.9809	0.9439	0.9965	-	0.9368
Cs+2E	25.36	2.577	**4**	0.0170	0.0474	0.0518	0.1816	0.5523	0.3726	0.2168	0.5875	0.9368	-

genotype	%N	std err	entry	_4	_3	_5	_6	_8	_9	_10	_1	_7	_2
Cs+2E	1.93	0.100	**4**	-	0.8329	0.7400	0.2754	0.2363	0.1392	0.0399	0.0374	0.1019	0.0184
Cs+1E	1.70	0.088	**3**	0.8329	-	1.0000	0.9999	0.9757	0.9430	0.9582	0.9564	0.6627	0.2352
Cs+3E	1.64	0.104	**5**	0.7400	1.0000	-	1.0000	0.9998	0.9989	0.9991	0.9989	0.9202	0.5589
Cs+4E	1.62	0.093	**6**	0.2754	0.9999	1.0000	-	1.0000	0.9998	0.9957	0.9931	0.9909	0.8389
Cs+6E	1.56	0.087	**8**	0.2363	0.9757	0.9998	1.0000	-	1.0000	1.0000	1.0000	0.9989	0.9042
Cs+7E	1.54	0.084	**9**	0.1392	0.9430	0.9989	0.9998	1.0000	-	1.0000	1.0000	0.9999	0.9591
Elongatum	1.51	0.104	**10**	0.0399	0.9582	0.9991	0.9957	1.0000	1.0000	-	1.0000	1.0000	0.9986
AgCs	1.50	0.110	**1**	0.0374	0.9564	0.9989	0.9931	1.0000	1.0000	1.0000	-	1.0000	0.9994
Cs+5E	1.47	0.095	**7**	0.1019	0.6627	0.9202	0.9909	0.9989	0.9999	1.0000	1.0000	-	0.9995
Cs	1.39	0.091	**2**	0.0184	0.2352	0.5589	0.8389	0.9042	0.9591	0.9986	0.9994	0.9995	-

genotype	TN	std err	entry	_3	_6	_9	_2	_8	_4	_1	_7	_10	_5
Cs+1E	0.585	0.0317	**3**	-	0.9596	0.9368	0.9210	0.3772	0.4257	0.2047	0.0329	0.0270	0.0265
Cs+4E	0.528	0.0317	**6**	0.9596	-	1.0000	1.0000	0.9839	0.9867	0.9133	0.4753	0.4296	0.4256
Cs+7E	0.524	0.0317	**9**	0.9368	1.0000	-	1.0000	0.9916	0.9930	0.9420	0.5396	0.4923	0.4881
Cs	0.522	0.0317	**2**	0.9210	1.0000	1.0000	-	0.9943	0.9953	0.9547	0.5757	0.5280	0.5237
Cs+6E	0.480	0.0317	**8**	0.3772	0.9839	0.9916	0.9943	-	1.0000	1.0000	0.9829	0.9741	0.9732
Cs+2E	0.479	0.0342	**4**	0.4257	0.9867	0.9930	0.9953	1.0000	-	1.0000	0.9881	0.9817	0.9810
AgCs	0.464	0.0317	**1**	0.2047	0.9133	0.9420	0.9547	1.0000	1.0000	-	0.9989	0.9977	0.9976
Cs+5E	0.430	0.0317	**7**	0.0329	0.4753	0.5396	0.5757	0.9829	0.9881	0.9989	-	1.0000	1.0000
Elongatum	0.427	0.0317	**10**	0.0270	0.4296	0.4923	0.5280	0.9741	0.9817	0.9977	1.0000	-	1.0000
Cs+3E	0.427	0.0317	**5**	0.0265	0.4256	0.4881	0.5237	0.9732	0.9810	0.9976	1.0000	1.0000	-

Table B.12: Genotype LS mean values and pairwise comparisons for aboveground biomass (weight), tissue N concentration (%N) and total plant N (TN) for addition genotypes at anthesis. Significant differences at the $p<0.05$ level are shaded.

genotype	grain wt (g)	std err	entry	_1	_2	_3	_4	_5	_6	_7	_8	_9	_10
Cs	16.30	1.717	**2**	0.0151	_	0.1438	0.8964	1.0000	0.1892	0.0418	0.0222	0.0394	<.0001
Cs+3E	16.01	1.717	**5**	0.0214	1.0000	0.1859	0.9304	_	0.2403	0.0573	0.0311	0.0528	<.0001
Cs+2E	12.04	2.325	**4**	0.8121	0.8964	0.9949	_	0.9304	0.9980	0.9360	0.8665	0.8810	0.0185
Cs+4E	9.68	1.717	**6**	0.9908	0.1892	1.0000	0.9980	0.2403	_	0.9997	0.9968	0.9970	0.0395
Cs+1E	9.37	1.717	**3**	0.9966	0.1438	_	0.9949	0.1859	1.0000	1.0000	0.9991	0.9990	0.0554
Cs+5E	8.13	1.717	**7**	1.0000	0.0418	1.0000	0.9360	0.0573	0.9997	_	1.0000	1.0000	0.1808
Cs+6E	7.57	1.717	**8**	1.0000	0.0222	0.9991	0.8665	0.0311	0.9968	1.0000	_	1.0000	0.2833
Cs+7E	7.42	1.983	**9**	1.0000	0.0394	0.9990	0.8810	0.0528	0.9970	1.0000	1.0000	_	0.4232
AgCs	7.24	1.717	**1**	_	0.0151	0.9966	0.8121	0.0214	0.9908	1.0000	1.0000	1.0000	0.3565
Elongatum	1.46	1.717	**10**	0.3565	<.0001	0.0554	0.0185	<.0001	0.0395	0.1808	0.2833	0.4232	_

genotype	grain %N	std err	entry	_1	_2	_3	_4	_5	_6	_7	_8	_9	_10
Cs+7E	4.327	0.2190	**9**	0.0001	<.0001	0.0372	0.0001	0.0006	0.0029	0.1518	0.0773	_	0.0157
Cs+5E	3.610	0.2318	**7**	0.0496	0.0311	0.9999	0.1038	0.6044	0.8990	_	1.0000	0.1518	0.2099
Cs+6E	3.552	0.1967	**8**	0.0270	0.0602	1.0000	0.0743	0.7545	0.9391	1.0000	_	0.0773	0.1702
Cs+1E	3.479	0.1897	**3**	0.0339	0.1342	_	0.1048	0.9092	0.9877	0.9999	1.0000	0.0372	0.1884
Cs+4E	3.235	0.1705	**6**	0.0764	0.7629	0.9877	0.3224	1.0000	_	0.8990	0.9391	0.0029	0.2635
Cs+3E	3.146	0.2084	**5**	0.3084	0.8930	0.9092	0.6748	_	1.0000	0.6044	0.7545	0.0006	0.4614
Cs	2.806	0.2140	**2**	0.8118	_	0.1342	0.9953	0.8930	0.7629	0.0311	0.0602	<.0001	0.7487
Cs+2E	2.477	0.2390	**4**	0.9454	0.9953	0.1048	_	0.6748	0.3224	0.1038	0.0743	0.0001	0.7204
AgCs	2.070	0.2895	**1**	_	0.8118	0.0339	0.9454	0.3084	0.0764	0.0496	0.0270	0.0001	0.8773
Elongatum	1.361	0.6634	**10**	0.8773	0.7487	0.1884	0.7204	0.4614	0.2635	0.2099	0.1702	0.0157	_

genotype	grain TN	std err	entry	_1	_2	_3	_4	_5	_6	_7	_8	_9	_10
Cs+3E	0.4381	0.03965	**5**	0.0017	0.9943	0.1127	0.8719	_	0.0130	0.0285	0.0273	0.5544	<.0001
Cs	0.3854	0.03965	**2**	0.0289	_	0.5851	0.9990	0.9943	0.1472	0.2572	0.2495	0.9690	<.0001
Cs+2E	0.3357	0.05368	**4**	0.4693	0.9990	0.9935	_	0.8719	0.8194	0.9176	0.9131	1.0000	0.0066
Cs+7E	0.3127	0.04578	**9**	0.5733	0.9690	0.9996	1.0000	0.5544	0.9110	0.9728	0.9705	_	0.0063
Cs+1E	0.2718	0.03965	**3**	0.8944	0.5851	_	0.9935	0.1127	0.9980	0.9999	0.9999	0.9996	0.0207
Cs+5E	0.2414	0.03965	**7**	0.9945	0.2572	0.9999	0.9176	0.0285	1.0000	_	1.0000	0.9728	0.0817
Cs+6E	0.2405	0.03965	**8**	0.9951	0.2495	0.9999	0.9131	0.0273	1.0000	1.0000	_	0.9705	0.0848
Cs+4E	0.2259	0.03965	**6**	0.9996	0.1472	0.9980	0.8194	0.0130	_	1.0000	1.0000	0.9110	0.1501
AgCs	0.1889	0.03965	**1**	_	0.0289	0.8944	0.4693	0.0017	0.9996	0.9945	0.9951	0.5733	0.4668
Elongatum	0.0603	0.04282	**10**	0.4668	<.0001	0.0207	0.0066	<.0001	0.1501	0.0817	0.0848	0.0063	_

Table B.13: Genotype LS mean values and pairwise comparisons for grain weight, grain N concentration (%N) and grain total N (TN) for addition genotypes at maturity. Significant differences at the p<0.05 level are shaded.

genotype	straw wt (g)	std err	entry	_1	_2	_3	_4	_5	_6	_7	_8	_9	_10
Elongatum	52.87	3.396	10	0.9678	0.1517	0.0143	0.0402	0.0045	0.9446	0.0013	0.2035	0.0240	_
AgCs	47.06	3.396	1	_	0.8315	0.2700	0.3665	0.1235	1.0000	0.0495	0.8938	0.3181	0.9678
Cs+4E	46.53	3.396	6	1.0000	0.8820	0.3285	0.4235	0.1577	_	0.0657	0.9312	0.3770	0.9446
Cs+6E	39.95	3.396	8	0.8938	1.0000	0.9865	0.9804	0.9078	0.9312	0.7202	_	0.9849	0.2035
Cs	39.27	3.396	2	0.8315	_	0.9951	0.9910	0.9491	0.8820	0.8025	1.0000	0.9939	0.1517
Cs+1E	34.85	3.396	3	0.2700	0.9951	_	1.0000	1.0000	0.3285	0.9989	0.9865	1.0000	0.0143
Cs+7E	34.36	3.922	9	0.3181	0.9939	1.0000	1.0000	1.0000	0.3770	0.9998	0.9849	_	0.0240
Cs+2E	33.55	4.599	4	0.3665	0.9910	1.0000	_	1.0000	0.4235	1.0000	0.9804	1.0000	0.0402
Cs+3E	33.02	3.396	5	0.1235	0.9491	1.0000	1.0000	_	0.1577	1.0000	0.9078	1.0000	0.0045
Cs+5E	31.21	3.396	7	0.0495	0.8025	0.9989	1.0000	1.0000	0.0657	_	0.7202	0.9998	0.0013

genotype	straw %N	std err*	entry	_1	_2	_3	_4	_5	_6	_7	_8	_9	_10
Cs+5E	0.6362	1.117	7	0.0203	0.8366	0.9730	0.4910	0.9985	0.5231	_	0.9557	0.4402	0.1635
Cs+3E	0.5731	1.110	5	0.0577	0.9978	1.0000	0.8183	_	0.9236	0.9985	1.0000	0.8727	0.2771
Cs+1E	0.5430	1.105	3	0.0855	1.0000	_	0.9278	1.0000	0.9904	0.9730	1.0000	0.9758	0.3343
Cs+6E	0.5365	1.107	8	0.1129	1.0000	1.0000	0.9501	1.0000	0.9953	0.9557	_	0.9853	0.3808
Cs	0.5135	1.112	2	0.2321	_	1.0000	0.9898	0.9978	0.9999	0.8366	1.0000	0.9988	0.5284
Cs+4E	0.4745	1.100	6	0.3064	0.9999	0.9904	0.9998	0.9236	_	0.5231	0.9953	1.0000	0.5898
Cs+7E	0.4601	1.122	9	0.5857	0.9988	0.9758	1.0000	0.8727	1.0000	0.4402	0.9853	_	0.7719
Cs+2E	0.4282	1.140	4	0.6769	0.9898	0.9278	_	0.8183	0.9998	0.4910	0.9501	1.0000	0.7344
AgCs	0.3131	1.135	1	_	0.2321	0.0855	0.6769	0.0577	0.3064	0.0203	0.1129	0.5857	0.9999
Elongatum	0.2851	1.243	10	0.9999	0.5284	0.3343	0.7344	0.2771	0.5898	0.1635	0.3808	0.7719	_

genotype	straw TN	std err*	entry	_1	_2	_3	_4	_5	_6	_7	_8	_9	_10
Cs+4E	0.2188	1.110	6	0.0799	1.0000	0.9978	0.2146	0.9981	_	0.9995	1.0000	0.7211	0.1530
Cs+6E	0.2177	1.117	8	0.1439	1.0000	0.9983	0.2807	0.9984	1.0000	0.9996	_	0.7280	0.2315
Cs	0.2126	1.122	2	0.2269	_	0.9997	0.3835	0.9997	1.0000	1.0000	1.0000	0.8169	0.3105
Cs+5E	0.1975	1.128	7	0.4692	1.0000	1.0000	0.6623	1.0000	0.9995	_	0.9996	0.9733	0.4958
Cs+3E	0.1940	1.121	5	0.4589	0.9997	1.0000	0.6817	_	0.9981	1.0000	0.9984	0.9859	0.4793
Cs+1E	0.1939	1.115	3	0.3986	0.9997	_	0.6485	1.0000	0.9978	1.0000	0.9983	0.9861	0.4274
Cs+7E	0.1642	1.134	9	0.9313	0.8169	0.9861	0.9920	0.9859	0.7211	0.9733	0.7280	_	0.8272
Cs+2E	0.1351	1.154	4	1.0000	0.3835	0.6485	_	0.6817	0.2146	0.6623	0.2807	0.9920	0.9699
AgCs	0.1237	1.148	1	_	0.2269	0.3986	1.0000	0.4589	0.0799	0.4692	0.1439	0.9313	0.9828
Elongatum	0.1005	1.267	10	0.9828	0.3105	0.4274	0.9699	0.4793	0.1530	0.4958	0.2315	0.8272	_

Table B.14: Genotype LS mean values and pairwise comparisons for straw weight, straw N concentration (%N) and straw total N (TN) for addition genotypes at maturity. Significant differences at the $p<0.05$ level are shaded. * Straw %N was analysed using a logarithmic transformation of the data. Therefore, the standard error is a percentage, rather than an absolute estimation of the error. To get one standard deviation on either side of the point estimate, multiply and divide the point estimate by the standard error, rather than adding and subtracting the standard error as would be done for the untransformed variables.

genotype	biomass (g)	std err	entry	_1	_2	_3	_4	_5	_6	_7	_8	_9	_10
Cs+4E	56.21	3.715	**6**	1.0000	1.0000	0.4163	0.7910	0.9318	_	0.0622	0.8148	0.2685	1.0000
Cs	55.58	3.715	**2**	1.0000	_	0.4946	0.8437	0.9610	1.0000	0.0839	0.8725	0.3274	1.0000
Elongatum	54.33	3.715	**10**	1.0000	1.0000	0.6535	0.9228	0.9905	1.0000	0.1447	0.9504	0.4607	_
AgCs	54.31	3.715	**1**	_	1.0000	0.6561	0.9238	0.9908	1.0000	0.1460	0.9513	0.4632	1.0000
Cs+3E	49.03	3.715	**5**	0.9908	0.9610	0.9953	0.9999	_	0.9318	0.7050	1.0000	0.9542	0.9905
Cs+6E	47.52	3.715	**8**	0.9513	0.8725	0.9998	1.0000	1.0000	0.8148	0.8626	_	0.9902	0.9504
Cs+2E	45.59	5.030	**4**	0.9238	0.8437	1.0000	_	0.9999	0.7910	0.9911	1.0000	0.9999	0.9228
Cs+1E	44.22	3.715	**3**	0.6561	0.4946	_	1.0000	0.9953	0.4163	0.9948	0.9998	1.0000	0.6535
Cs+7E	41.77	4.289	**9**	0.4632	0.3274	1.0000	0.9999	0.9542	0.2685	1.0000	0.9902	_	0.4607
Cs+5E	39.34	3.715	**7**	0.1460	0.0839	0.9948	0.9911	0.7050	0.0622	_	0.8626	1.0000	0.1447

genotype	HI	std err	entry	_1	_2	_3	_4	_5	_6	_7	_8	_9	_10
Cs+3E	0.3348	0.02835	**5**	0.0003	0.9790	0.0543	0.8620	_	0.0023	0.1419	0.0041	0.0203	<.0001
Cs	0.2894	0.02835	**2**	0.0134	_	0.5136	0.9998	0.9790	0.0632	0.7742	0.0974	0.2518	<.0001
Cs+2E	0.2605	0.03839	**4**	0.2602	0.9998	0.9716	_	0.8620	0.5446	0.9974	0.6447	0.8221	0.0004
Cs+5E	0.2201	0.02835	**7**	0.5744	0.7742	1.0000	0.9974	0.1419	0.8958	_	0.9500	0.9923	0.0006
Cs+1E	0.2039	0.02835	**3**	0.8232	0.5136	_	0.9716	0.0543	0.9855	1.0000	0.9960	0.9998	0.0021
Cs+7E	0.1777	0.03274	**9**	0.9954	0.2518	0.9998	0.8221	0.0203	1.0000	0.9923	1.0000	_	0.0345
Cs+6E	0.1681	0.02835	**8**	0.9990	0.0974	0.9960	0.6447	0.0041	1.0000	0.9500	_	1.0000	0.0313
Cs+4E	0.1609	0.02835	**6**	0.9999	0.0632	0.9855	0.5446	0.0023	_	0.8958	1.0000	1.0000	0.0503
AgCs	0.1382	0.02835	**1**	_	0.0134	0.8232	0.2602	0.0003	0.9999	0.5744	0.9990	0.9954	0.1883
Elongatum	0.0288	0.02835	**10**	0.1883	<.0001	0.0021	0.0004	<.0001	0.0503	0.0006	0.0313	0.0345	_

genotype	NHI	std err	entry	_1	_2	_3	_4	_5	_6	_7	_8	_9	_10
Cs+3E	0.7111	0.05329	**5**	0.2146	0.9993	0.7548	1.0000	_	0.0074	0.7177	0.2764	0.9727	<.0001
Cs+2E	0.6701	0.07215	**4**	0.7439	1.0000	0.9895	_	1.0000	0.1365	0.9852	0.8078	0.9999	<.0001
Cs	0.6571	0.05329	**2**	0.6381	_	0.9878	1.0000	0.9993	0.0558	0.9819	0.7242	1.0000	<.0001
Cs+7E	0.6152	0.06153	**9**	0.9521	1.0000	1.0000	0.9999	0.9727	0.2916	1.0000	0.9747	_	0.0001
Cs+1E	0.5783	0.05329	**3**	0.9958	0.9878	_	0.9895	0.7548	0.4623	1.0000	0.9988	1.0000	0.0002
Cs+5E	0.5737	0.05329	**7**	0.9975	0.9819	1.0000	0.9852	0.7177	0.5024	_	0.9994	1.0000	0.0002
Cs+6E	0.5205	0.05329	**8**	1.0000	0.7242	0.9988	0.8078	0.2764	0.9076	0.9994	_	0.9747	0.0021
AgCs	0.5104	0.05329	**1**	_	0.6381	0.9958	0.7439	0.2146	0.9472	0.9975	1.0000	0.9521	0.0032
Cs+4E	0.4117	0.05329	**6**	0.9472	0.0558	0.4623	0.1365	0.0074	_	0.5024	0.9076	0.2916	0.1066
Elongatum	0.1772	0.05756	**10**	0.0032	<.0001	0.0002	<.0001	<.0001	0.1066	0.0002	0.0021	0.0001	_

Table B.15: Genotype LS mean values and pairwise comparisons for aboveground biomass, harvest index (HI) and nitrogen harvest index (NHI) at maturity. Significant differences at the $p<0.05$ level are shaded.

genotype	N up	std err	entry	_1	_2	_3	_4	_5	_6	_7	_8	_9	_10
Cs+3E	0.6070	0.03777	**5**	0.0047	1.0000	0.1187	0.8107	_	0.1493	0.0209	0.0632	0.2855	0.0020
Cs	0.5873	0.03777	**2**	0.0144	_	0.2547	0.9359	1.0000	0.3071	0.0563	0.1498	0.4885	0.0062
Cs+2E	0.5013	0.05114	**4**	0.7327	0.9359	0.9981	_	0.8107	0.9992	0.9280	0.9889	0.9999	0.5285
Cs+7E	0.4621	0.04361	**9**	0.9488	0.4885	1.0000	0.9999	0.2855	1.0000	0.9975	1.0000	_	0.8147
Cs+4E	0.4553	0.03777	**6**	0.9538	0.3071	1.0000	0.9992	0.1493	_	0.9986	1.0000	1.0000	0.8139
Cs+1E	0.4498	0.03777	**3**	0.9728	0.2547	_	0.9981	0.1187	1.0000	0.9995	1.0000	1.0000	0.8622
Cs+6E	0.4357	0.03777	**8**	0.9954	0.1498	1.0000	0.9889	0.0632	1.0000	1.0000	_	1.0000	0.9485
Cs+5E	0.4135	0.03777	**7**	1.0000	0.0563	0.9995	0.9280	0.0209	0.9986	_	1.0000	0.9975	0.9957
AgCs	0.3869	0.03777	**1**	_	0.0144	0.9728	0.7327	0.0047	0.9538	1.0000	0.9954	0.9488	1.0000
Elongatum	0.3632	0.04080	**10**	1.0000	0.0062	0.8622	0.5285	0.0020	0.8139	0.9957	0.9485	0.8147	_

genotype	N ut	std err	entry	_1	_2	_3	_4	_5	_6	_7	_8	_9	_10
Cs	29.61	2.689	**2**	0.2248	_	0.2112	0.9911	0.9999	0.0459	0.2258	0.0303	0.0364	<.0001
Cs+3E	27.45	2.689	**5**	0.5530	0.9999	0.5317	0.9999	_	0.1746	0.5547	0.1247	0.1320	<.0001
Cs+2E	25.08	3.642	**4**	0.9661	0.9911	0.9611	_	0.9999	0.7323	0.9665	0.6489	0.6094	0.0036
Cs+5E	19.57	2.689	**7**	1.0000	0.2258	1.0000	0.9665	0.5547	0.9994	_	0.9973	0.9924	0.0246
AgCs	19.56	2.689	**1**	_	0.2248	1.0000	0.9661	0.5530	0.9995	1.0000	0.9974	0.9925	0.0247
Cs+1E	19.44	2.689	**3**	1.0000	0.2112	_	0.9611	0.5317	0.9996	1.0000	0.9980	0.9940	0.0269
Cs+4E	16.93	2.689	**6**	0.9995	0.0459	0.9996	0.7323	0.1746	_	0.9994	1.0000	1.0000	0.1319
Cs+6E	16.34	2.689	**8**	0.9974	0.0303	0.9980	0.6489	0.1247	1.0000	0.9973	_	1.0000	0.1816
Cs+7E	15.56	3.106	**9**	0.9925	0.0364	0.9940	0.6094	0.1320	1.0000	0.9924	1.0000	_	0.3621
Elongatum	5.47	2.905	**10**	0.0247	<.0001	0.0269	0.0036	<.0001	0.1319	0.0246	0.1816	0.3621	_

Table B.16: Genotype LS mean values and pairwise comparisons for total N uptake (N up) and N utilization efficiency (Nut) at maturity. Significant differences at the $p<0.05$ level are shaded.

genotype	weight (g)	std err	entry	_9	_3	_8	_1	_2	_6	_7	_5	_4
SS259	0.79	0.090	**9**	_	0.9999	0.9997	0.9599	0.9443	0.9275	0.5996	0.3601	0.0965
03JP019	0.73	0.090	**3**	0.9999	_	1.0000	0.9986	0.9973	0.9955	0.8706	0.6627	0.2588
PI550713	0.72	0.090	**8**	0.9997	1.0000	_	0.9995	0.9990	0.9981	0.9078	0.7233	0.3070
03JP004	0.64	0.090	**1**	0.9599	0.9986	0.9995	_	1.0000	1.0000	0.9975	0.9664	0.6851
03JP016	0.63	0.090	**2**	0.9443	0.9973	0.9990	1.0000	_	1.0000	0.9988	0.9771	0.7286
03JP036	0.62	0.090	**6**	0.9275	0.9955	0.9981	1.0000	1.0000	_	0.9994	0.9841	0.7648
03JP039	0.54	0.090	**7**	0.5996	0.8706	0.9078	0.9975	0.9988	0.9994	_	1.0000	0.9803
03JP026	0.50	0.090	**5**	0.3601	0.6627	0.7233	0.9664	0.9771	0.9841	1.0000	_	0.9991
03JP022	0.41	0.090	**4**	0.0965	0.2588	0.3070	0.6851	0.7286	0.7648	0.9803	0.9991	_

genotype	%N	std err	entry	_2	_4	_1	_5	_7	_3	_9	_6	_8
03JP016	5.826	0.1094	**2**	_	0.9828	0.9665	0.9289	0.5839	0.3635	0.1216	0.1029	0.0905
03JP022	5.669	0.1094	**4**	0.9828	_	1.0000	1.0000	0.9899	0.9330	0.6518	0.6035	0.5667
03JP004	5.651	0.1094	**1**	0.9665	1.0000	_	1.0000	0.9959	0.9609	0.7260	0.6801	0.6443
03JP026	5.626	0.1094	**5**	0.9289	1.0000	1.0000	_	0.9991	0.9842	0.8174	0.7779	0.7459
03JP039	5.524	0.1094	**7**	0.5839	0.9899	0.9959	0.9991	_	1.0000	0.9913	0.9857	0.9798
03JP019	5.471	0.1094	**3**	0.3635	0.9330	0.9609	0.9842	1.0000	_	0.9997	0.9993	0.9987
SS259	5.383	0.1094	**9**	0.1216	0.6518	0.7260	0.8174	0.9913	0.9997	_	1.0000	1.0000
03JP036	5.372	0.1094	**6**	0.1029	0.6035	0.6801	0.7779	0.9857	0.9993	1.0000	_	1.0000
PI550713	5.363	0.1094	**8**	0.0905	0.5667	0.6443	0.7459	0.9798	0.9987	1.0000	1.0000	_

genotype	TN (g)	std err	entry	_9	_3	_8	_2	_1	_6	_7	_5	_4
SS259	0.0435	0.00501	**9**	_	1.0000	0.9998	0.9821	0.9779	0.9326	0.6896	0.5355	0.1211
03JP019	0.0414	0.00501	**3**	1.0000	_	1.0000	0.9981	0.9974	0.9854	0.8524	0.7255	0.2227
PI550713	0.0396	0.00501	**8**	0.9998	1.0000	_	0.9999	0.9999	0.9981	0.9440	0.8630	0.3523
03JP016	0.0363	0.00501	**2**	0.9821	0.9981	0.9999	_	1.0000	1.0000	0.9973	0.9842	0.6545
03JP004	0.0360	0.00501	**1**	0.9779	0.9974	0.9999	1.0000	_	1.0000	0.9981	0.9874	0.6774
03JP036	0.0344	0.00501	**6**	0.9326	0.9854	0.9981	1.0000	1.0000	_	0.9999	0.9979	0.8099
03JP039	0.0308	0.00501	**7**	0.6896	0.8524	0.9440	0.9973	0.9981	0.9999	_	1.0000	0.9754
03JP026	0.0292	0.00501	**5**	0.5355	0.7255	0.8630	0.9842	0.9874	0.9979	1.0000	_	0.9949
03JP022	0.0232	0.00501	**4**	0.1211	0.2227	0.3523	0.6545	0.6774	0.8099	0.9754	0.9949	_

Table B.17: LS mean values and pairwise comparisons for aboveground biomass (weight) and tissue N concentration (%N) and total plant N (TN) for perennial lines at the 6leaf stage. Significant differences at the $p<0.05$ level are shaded.

genotype	weight (g)	std err	entry	_5	_2	_9	_1	_6	_4	_3	_8	_7
03JP026	40.63	2.087	**5**	_	0.9927	0.9855	0.9168	0.8700	0.7624	0.3536	0.2193	0.0393
03JP016	38.05	2.060	**2**	0.9927	_	1.0000	1.0000	0.9991	0.9967	0.8293	0.7359	0.2283
SS259	37.59	2.163	**9**	0.9855	1.0000	_	1.0000	0.9999	0.9994	0.8686	0.8431	0.2404
03JP004	36.77	2.076	**1**	0.9168	1.0000	1.0000	_	1.0000	1.0000	0.9682	0.9366	0.4788
03JP036	35.81	2.817	**6**	0.8700	0.9991	0.9999	1.0000	_	1.0000	0.9991	0.9968	0.8846
03JP022	35.75	2.052	**4**	0.7624	0.9967	0.9994	1.0000	1.0000	_	0.9954	0.9905	0.6569
03JP019	33.18	2.339	**3**	0.3536	0.8293	0.8686	0.9682	0.9991	0.9954	_	1.0000	0.9864
PI550713	33.07	2.052	**8**	0.2193	0.7359	0.8431	0.9366	0.9968	0.9905	1.0000	_	0.9888
03JP039	30.23	2.195	**7**	0.0393	0.2283	0.2404	0.4788	0.8846	0.6569	0.9864	0.9888	_

genotype	%N	std err*	entry	_7	_8	_3	_6	_2	_1	_4	_9	_5
03JP039	1.658	1.061	**7**	_	0.8590	0.5166	0.6267	0.2555	0.2226	0.0690	0.0231	<.0001
PI550713	1.450	1.064	**8**	0.8590	_	1.0000	0.9996	0.9966	0.9884	0.8278	0.8366	0.0022
03JP019	1.412	1.064	**3**	0.5166	1.0000	_	1.0000	0.9999	0.9995	0.9679	0.9097	0.0096
03JP036	1.367	1.076	**6**	0.6267	0.9996	1.0000	_	1.0000	1.0000	0.9989	0.9979	0.0337
03JP016	1.357	1.055	**2**	0.2555	0.9966	0.9999	1.0000	_	1.0000	0.9984	0.9945	0.0115
03JP004	1.342	1.055	**1**	0.2226	0.9884	0.9995	1.0000	1.0000	_	0.9997	0.9987	0.0168
03JP022	1.285	1.056	**4**	0.0690	0.8278	0.9679	0.9989	0.9984	0.9997	_	1.0000	0.0845
SS259	1.269	1.059	**9**	0.0231	0.8366	0.9097	0.9979	0.9945	0.9987	1.0000	_	0.1700
03JP026	1.019	1.056	**5**	<.0001	0.0022	0.0096	0.0337	0.0115	0.0168	0.0845	0.1700	_

genotype	TN (g)	std err	entry	_2	_1	_6	_7	_9	_3	_5	_4	_8
03JP016	0.5393	0.02813	**2**	_	1.0000	1.0000	0.9888	0.9842	0.8760	0.6560	0.5872	0.1790
03JP004	0.5279	0.02813	**1**	1.0000	_	1.0000	0.9991	0.9984	0.9600	0.8246	0.7684	0.3072
03JP036	0.5253	0.03038	**6**	1.0000	1.0000	_	0.9997	0.9994	0.9769	0.8811	0.8363	0.3964
03JP039	0.5016	0.02813	**7**	0.9888	0.9991	0.9997	_	1.0000	0.9998	0.9925	0.9846	0.7223
SS259	0.4995	0.02813	**9**	0.9842	0.9984	0.9994	1.0000	_	0.9999	0.9950	0.9891	0.7535
03JP019	0.4796	0.03038	**3**	0.8760	0.9600	0.9769	0.9998	0.9999	_	1.0000	1.0000	0.9656
03JP026	0.4661	0.02813	**5**	0.6560	0.8246	0.8811	0.9925	0.9950	1.0000	_	1.0000	0.9948
03JP022	0.4619	0.02813	**4**	0.5872	0.7684	0.8363	0.9846	0.9891	1.0000	1.0000	_	0.9979
PI550713	0.4325	0.02813	**8**	0.1790	0.3072	0.3964	0.7223	0.7535	0.9656	0.9948	0.9979	_

Table B.18: LS mean values and pairwise comparisons for aboveground biomass (weight), tissue N concentration (%N) and total plant N (TN) for perennial lines at anthesis. Significant differences at the p<0.05 level are shaded.

genotype	grain wt (g)	STDERR	entry	_2	_8	_1	_3	_9	_5	_4	_6	_7
03JP016	18.04	1.637	**2**	_	0.9999	0.9993	0.9980	0.9644	0.3714	0.2824	0.0400	0.0085
PI550713	16.90	1.637	**8**	0.9999	_	1.0000	1.0000	0.9992	0.6900	0.5832	0.1323	0.0345
03JP004	16.51	1.769	**1**	0.9993	1.0000	_	1.0000	0.9999	0.8226	0.7351	0.2305	0.0727
03JP019	16.34	1.637	**3**	0.9980	1.0000	1.0000	_	1.0000	0.8282	0.7380	0.2178	0.0641
SS259	15.39	1.637	**9**	0.9644	0.9992	0.9999	1.0000	_	0.9654	0.9266	0.4356	0.1635
03JP026	12.76	1.637	**5**	0.3714	0.6900	0.8226	0.8282	0.9654	_	1.0000	0.9802	0.8068
03JP022	12.38	1.637	**4**	0.2824	0.5832	0.7351	0.7380	0.9266	1.0000	_	0.9934	0.8830
03JP036	10.36	1.637	**6**	0.0400	0.1323	0.2305	0.2178	0.4356	0.9802	0.9934	_	0.9998
03JP039	9.08	1.637	**7**	0.0085	0.0345	0.0727	0.0641	0.1635	0.8068	0.8830	0.9998	_

genotype	grain %N	STDERR	entry	_7	_6	_4	_1	_9	_8	_5	_2	_3
03JP039	3.993	0.2145	**7**	_	0.7932	0.0745	0.0103	0.0040	0.0010	0.0005	0.0002	<.0001
03JP036	3.504	0.2145	**6**	0.7932	_	0.8684	0.3881	0.2528	0.1062	0.0671	0.0302	0.0154
03JP022	3.061	0.2145	**4**	0.0745	0.8684	_	0.9945	0.9792	0.8672	0.7693	0.5736	0.4171
03JP004	2.793	0.2316	**1**	0.0103	0.3881	0.9945	_	1.0000	0.9997	0.9979	0.9803	0.9360
SS259	2.744	0.2145	**9**	0.0040	0.2528	0.9792	1.0000	_	1.0000	0.9995	0.9909	0.9613
PI550713	2.618	0.2145	**8**	0.0010	0.1062	0.8672	0.9997	1.0000	_	1.0000	0.9999	0.9979
03JP026	2.559	0.2145	**5**	0.0005	0.0671	0.7693	0.9979	0.9995	1.0000	_	1.0000	0.9998
03JP016	2.465	0.2145	**2**	0.0002	0.0302	0.5736	0.9803	0.9909	0.9999	1.0000	_	1.0000
03JP019	2.392	0.2145	**3**	<.0001	0.0154	0.4171	0.9360	0.9613	0.9979	0.9998	1.0000	_

genotype	grain TN (g)	STDERR	entry	_1	_2	_8	_9	_3	_4	_7	_6	_5
03JP004	0.4519	0.04842	**1**	_	1.0000	1.0000	0.9973	0.9517	0.9464	0.8079	0.6988	0.6009
03JP016	0.4399	0.04483	**2**	1.0000	_	1.0000	0.9995	0.9767	0.9734	0.8679	0.7693	0.6736
PI550713	0.4357	0.04483	**8**	1.0000	1.0000	_	0.9998	0.9844	0.9819	0.8949	0.8061	0.7158
SS259	0.4013	0.04483	**9**	0.9973	0.9995	0.9998	_	0.9999	0.9999	0.9945	0.9790	0.9521
03JP019	0.3724	0.04483	**3**	0.9517	0.9767	0.9844	0.9999	_	1.0000	1.0000	0.9996	0.9978
03JP022	0.3709	0.04483	**4**	0.9464	0.9734	0.9819	0.9999	1.0000	_	1.0000	0.9997	0.9982
03JP039	0.3473	0.04483	**7**	0.8079	0.8679	0.8949	0.9945	1.0000	1.0000	_	1.0000	1.0000
03JP036	0.3349	0.04483	**6**	0.6988	0.7693	0.8061	0.9790	0.9996	0.9997	1.0000	_	1.0000
03JP026	0.3250	0.04483	**5**	0.6009	0.6736	0.7158	0.9521	0.9978	0.9982	1.0000	1.0000	_

Table B.19: LS mean values and pairwise comparisons for grain weight, grain N concentration (%N) and grain total N (TN) for perennial lines at maturity. Significant differences at the p<0.05 level are shaded.

genotype	straw wt (g)	STDERR	entry	_3	_5	_8	_4	_9	_1	_2	_6	_7
03JP019	51.43	3.149	**3**	_	0.9999	0.9859	0.9394	0.8510	0.9655	0.4846	0.1083	0.0081
03JP026	49.08	3.067	**5**	0.9999	_	1.0000	0.9945	0.9966	0.9936	0.9006	0.1628	0.0641
PI550713	47.43	2.875	**8**	0.9859	1.0000	_	1.0000	0.9999	1.0000	0.9682	0.4186	0.0913
03JP022	45.67	2.950	**4**	0.9394	0.9945	1.0000	_	1.0000	1.0000	0.9993	0.6238	0.2967
SS259	45.43	3.014	**9**	0.8510	0.9966	0.9999	1.0000	_	1.0000	0.9994	0.7689	0.2732
03JP004	45.42	3.577	**1**	0.9655	0.9936	1.0000	1.0000	1.0000	_	0.9999	0.7444	0.4983
03JP016	42.95	2.919	**2**	0.4846	0.9006	0.9682	0.9993	0.9994	0.9999	_	0.9678	0.6411
03JP036	38.14	2.933	**6**	0.1083	0.1628	0.4186	0.6238	0.7689	0.7444	0.9678	_	0.9993
03JP039	35.50	2.859	**7**	0.0081	0.0641	0.0913	0.2967	0.2732	0.4983	0.6411	0.9993	_

genotype	straw %N	STDERR*	entry	_7	_4	_6	_5	_8	_1	_2	_9	_3
03JP039	0.8256	1.120	**7**	_	0.6340	0.5539	0.4650	0.0465	0.0028	0.0002	<.0001	<.0001
03JP022	0.6120	1.120	**4**	0.6340	_	1.0000	1.0000	0.8975	0.2923	0.0576	0.0212	0.0169
03JP036	0.6003	1.120	**6**	0.5539	1.0000	_	1.0000	0.9364	0.3554	0.0776	0.0296	0.0237
03JP026	0.5875	1.120	**5**	0.4650	1.0000	1.0000	_	0.9665	0.4337	0.1066	0.0425	0.0342
PI550713	0.4903	1.120	**8**	0.0465	0.8975	0.9364	0.9665	_	0.9730	0.6886	0.4428	0.3925
03JP004	0.4089	1.130	**1**	0.0028	0.2923	0.3554	0.4337	0.9730	_	0.9994	0.9854	0.9766
03JP016	0.3684	1.120	**2**	0.0002	0.0576	0.0776	0.1066	0.6886	0.9994	_	1.0000	0.9999
SS259	0.3470	1.120	**9**	<.0001	0.0212	0.0296	0.0425	0.4428	0.9854	1.0000	_	1.0000
03JP019	0.3425	1.120	**3**	<.0001	0.0169	0.0237	0.0342	0.3925	0.9766	0.9999	1.0000	_

genotype	straw TN (g)	STDERR*	entry	_7	_5	_4	_8	_6	_3	_1	_9	_2
03JP039	0.2822	1.103	**7**	_	0.9999	0.9999	0.8566	0.6650	0.0484	0.0626	0.0039	0.0030
03JP026	0.2621	1.111	**5**	0.9999	_	1.0000	0.9939	0.9035	0.3390	0.0780	0.0610	0.0416
03JP022	0.2620	1.106	**4**	0.9999	1.0000	_	0.9926	0.9017	0.2915	0.0878	0.0461	0.0315
PI550713	0.2303	1.104	**8**	0.8566	0.9939	0.9926	_	1.0000	0.6764	0.6262	0.1861	0.1610
03JP036	0.2171	1.106	**6**	0.6650	0.9035	0.9017	1.0000	_	0.9437	0.7338	0.5233	0.4537
03JP019	0.1789	1.114	**3**	0.0484	0.3390	0.2915	0.6764	0.9437	_	1.0000	0.9952	0.9932
03JP004	0.1687	1.130	**1**	0.0626	0.0780	0.0878	0.6262	0.7338	1.0000	_	1.0000	1.0000
SS259	0.1595	1.109	**9**	0.0039	0.0610	0.0461	0.1861	0.5233	0.9952	1.0000	_	1.0000
03JP016	0.1584	1.105	**2**	0.0030	0.0416	0.0315	0.1610	0.4537	0.9932	1.0000	1.0000	_

Table B.20: LS mean values and pairwise comparisons for straw weight, straw N concentration (%N) and straw total N (TN) for perennial lines at maturity. Significant differences at the p<0.05 level are shaded. * Straw %N and straw TN were analysed using a logarithmic transformation of the data. Therefore, the standard error is a percentage, rather than an absolute estimation of the error. To get one standard deviation on either side of the point estimate, multiply and divide the point estimate by the standard error, rather than adding and subtracting the standard error as would be done for the untransformed variables.

genotype	biomass (g)	STDERR	entry	_1	_3	_5	_8	_4	_2	_9	_6	_7
03JP004	65.86	4.120	**1**	_	1.0000	1.0000	0.9999	0.9765	0.9635	0.9291	0.1421	0.0063
03JP019	64.84	3.814	**3**	1.0000	_	1.0000	1.0000	0.9907	0.9837	0.9621	0.1669	0.0069
03JP026	64.36	3.814	**5**	1.0000	1.0000	_	1.0000	0.9953	0.9910	0.9764	0.1997	0.0090
PI550713	63.15	3.814	**8**	0.9999	1.0000	1.0000	_	0.9995	0.9987	0.9949	0.3035	0.0173
03JP022	59.87	3.814	**4**	0.9765	0.9907	0.9953	0.9995	_	1.0000	1.0000	0.6848	0.0851
03JP016	59.41	3.814	**2**	0.9635	0.9837	0.9910	0.9987	1.0000	_	1.0000	0.7372	0.1037
SS259	58.60	3.814	**9**	0.9291	0.9621	0.9764	0.9949	1.0000	1.0000	_	0.8211	0.1450
03JP036	50.18	3.814	**6**	0.1421	0.1669	0.1997	0.3035	0.6848	0.7372	0.8211	_	0.9478
03JP039	43.59	3.814	**7**	0.0063	0.0069	0.0090	0.0173	0.0851	0.1037	0.1450	0.9478	_

genotype	HI	STDERR	entry	_2	_8	_3	_9	_1	_6	_4	_7	_5
03JP016	0.2791	0.02272	**2**	_	0.9931	0.9598	0.8745	0.8162	0.2039	0.1729	0.1121	0.0763
PI550713	0.2508	0.02272	**8**	0.9931	_	1.0000	0.9997	0.9982	0.7211	0.6697	0.5378	0.4313
03JP019	0.2416	0.02272	**3**	0.9598	1.0000	_	1.0000	0.9999	0.8726	0.8354	0.7247	0.6195
SS259	0.2326	0.02272	**9**	0.8745	0.9997	1.0000	_	1.0000	0.9589	0.9400	0.8712	0.7909
03JP004	0.2266	0.02454	**1**	0.8162	0.9982	0.9999	1.0000	_	0.9890	0.9818	0.9495	0.9028
03JP036	0.1950	0.02272	**6**	0.2039	0.7211	0.8726	0.9589	0.9890	_	1.0000	1.0000	1.0000
03JP022	0.1924	0.02272	**4**	0.1729	0.6697	0.8354	0.9400	0.9818	1.0000	_	1.0000	1.0000
03JP039	0.1859	0.02272	**7**	0.1121	0.5378	0.7247	0.8712	0.9495	1.0000	1.0000	_	1.0000
03JP026	0.1807	0.02272	**5**	0.0763	0.4313	0.6195	0.7909	0.9028	1.0000	1.0000	1.0000	_

genotype	NHI	STDERR	entry	_2	_9	_1	_8	_3	_4	_6	_7	_5
03JP016	0.7007	0.05374	**2**	_	0.9998	0.9885	0.9647	0.9458	0.2166	0.1293	0.1271	0.0258
SS259	0.6615	0.05374	**9**	0.9998	_	0.9999	0.9994	0.9984	0.5033	0.3495	0.3450	0.0962
03JP004	0.6253	0.05804	**1**	0.9885	0.9999	_	1.0000	1.0000	0.8350	0.7000	0.6952	0.3092
PI550713	0.6139	0.05374	**8**	0.9647	0.9994	1.0000	_	1.0000	0.8747	0.7476	0.7429	0.3378
03JP019	0.6072	0.05374	**3**	0.9458	0.9984	1.0000	1.0000	_	0.9088	0.7980	0.7937	0.3893
03JP022	0.5041	0.05374	**4**	0.2166	0.5033	0.8350	0.8747	0.9088	_	1.0000	1.0000	0.9923
03JP036	0.4852	0.05374	**6**	0.1293	0.3495	0.7000	0.7476	0.7980	1.0000	_	1.0000	0.9992
03JP039	0.4846	0.05374	**7**	0.1271	0.3450	0.6952	0.7429	0.7937	1.0000	1.0000	_	0.9993
03JP026	0.4361	0.05374	**5**	0.0258	0.0962	0.3092	0.3378	0.3893	0.9923	0.9992	0.9993	_

Table B.21: LS mean values and pairwise comparisons for aboveground biomass, harvest index (HI) and nitrogen harvest index (NHI) at maturity. Significant differences at the $p<0.05$ level are shaded.

genotype	N up	STDERR	entry	_4	_7	_8	_1	_5	_2	_6	_9	_3
03JP022	0.6868	0.03917	**4**	_	1.0000	1.0000	1.0000	0.9916	0.8488	0.7856	0.3308	0.2558
03JP039	0.6693	0.03917	**7**	1.0000	_	1.0000	1.0000	0.9996	0.9558	0.9236	0.5261	0.4314
PI550713	0.6667	0.03917	**8**	1.0000	1.0000	_	1.0000	0.9998	0.9649	0.9371	0.5568	0.4607
03JP004	0.6615	0.04231	**1**	1.0000	1.0000	1.0000	_	1.0000	0.9837	0.9677	0.6678	0.5751
03JP026	0.6366	0.03917	**5**	0.9916	0.9996	0.9998	1.0000	_	0.9996	0.9983	0.8744	0.8046
03JP016	0.6035	0.03917	**2**	0.8488	0.9558	0.9649	0.9837	0.9996	_	1.0000	0.9946	0.9847
03JP036	0.5966	0.03917	**6**	0.7856	0.9236	0.9371	0.9677	0.9983	1.0000	_	0.9982	0.9936
SS259	0.5565	0.03917	**9**	0.3308	0.5261	0.5568	0.6678	0.8744	0.9946	0.9982	_	1.0000
03JP019	0.5483	0.03917	**3**	0.2558	0.4314	0.4607	0.5751	0.8046	0.9847	0.9936	1.0000	_

genotype	N ut	STDERR	entry	_3	_2	_9	_8	_1	_4	_5	_6	_7
03JP019	36.92	4.538	**3**	_	0.9496	0.7136	0.6073	0.5554	0.1131	0.0975	0.0379	0.0131
03JP016	29.12	4.538	**2**	0.9496	_	0.9998	0.9986	0.9956	0.7554	0.7163	0.4681	0.2527
SS259	25.69	4.538	**9**	0.7136	0.9998	_	1.0000	1.0000	0.9640	0.9506	0.8056	0.5695
PI550713	24.63	4.538	**8**	0.6073	0.9986	1.0000	_	1.0000	0.9861	0.9792	0.8827	0.6778
03JP004	23.61	4.902	**1**	0.5554	0.9956	1.0000	1.0000	_	0.9967	0.9945	0.9492	0.8098
03JP022	18.34	4.538	**4**	0.1131	0.7554	0.9640	0.9861	0.9967	_	1.0000	1.0000	0.9955
03JP026	17.92	4.538	**5**	0.0975	0.7163	0.9506	0.9792	0.9945	1.0000	_	1.0000	0.9975
03JP036	15.48	4.538	**6**	0.0379	0.4681	0.8056	0.8827	0.9492	1.0000	1.0000	_	1.0000
03JP039	13.04	4.538	**7**	0.0131	0.2527	0.5695	0.6778	0.8098	0.9955	0.9975	1.0000	_

Table B.22: LS mean values and pairwise comparisons for total N uptake (N up) and N utilization efficiency (Nut) at maturity. Significant differences at the $p<0.05$ level are shaded.

Appendix C

Wheat grower survey summary tables

Wheat Production in Washington

This document reports responses to a mail survey of Washington wheat growers that was conducted from January through March, 2006. The survey's objective was to identify wheat growers' priorities in wheat breeding and related research programs at Washington State University (WSU). The survey was designed and sponsored by the Winter and Spring Wheat Breeding Programs in the Department of Crop and Soil Sciences and faculty in the Department of Community and Rural Sociology. The survey was conducted with the cooperation of WSU's Social and Economic Sciences Research Center (SESRC).

A total of 1,374 names were drawn from the list of members of the Washington Association of Wheat Growers. Three hundred and seven (307) individuals were excluded from the sample because of ineligibility, bad addresses, and other reasons. The result was a corrected sample of 1,067 growers. Of these, 553 wheat growers returned completed questionnaires. The completion rate for the survey was 51.8 percent.

Of those farmers who responded to the survey, less than 10 percent reported that they manage an agricultural business in addition to running their farm. Only 2 percent stated that they hire a management company to help run their farm. Respondents ranged in age from 26 to 96 years with an average age of 57 years. The average number of years of farming experience (as either a farm owner or manager) was 32 years. Over 96 percent of respondents were male.

Content

PART I. EXPERIENCE WITH WASHINGTON STATE UNIVERSITY (WSU)

Growers' frequency of contact with WSU representatives during 2003 – 2005.

	At least once a week (%)	At least once a month but less than every week (%)	More than once a year but less than once a month (%)	About once a year or less (%)	Not at all (%)
WSU Extension Specialists	0.4	7.4	33.8	31.0	27.3
WSU-Pullman Researchers	0.4	3.1	22.7	33.4	40.5
WSU Administration	0.4	1.9	6.4	13.5	77.8

Growers' satisfaction with their contact with WSU representatives during 2003 – 2005. (*Data are for growers who had contact with WSU representatives during 2003 – 2005)*

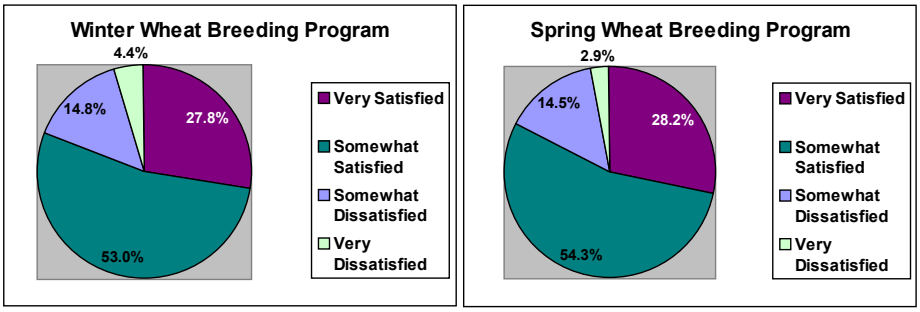
	Very satisfied (%)	Somewhat satisfied (%)	Somewhat dissatisfied (%)	Very dissatisfied (%)
WSU Extension Specialists	57.8	33.4	5.2	3.6
WSU-Pullman Researchers	52.9	37.2	6.3	3.6
WSU Administration	32.1	45.7	13.6	8.6

Growers' perceptions of the level of success of WSU representatives at serving the needs of wheat growers during 2003 – 2005.

	Very successful (%)	Somewhat successful (%)	Somewhat unsuccessful (%)	Very unsuccessful (%)	Does not apply (%)
WSU Extension Specialists	29.3	45.3	10.4	2.8	12.2
WSU-Pullman Researchers	27.0	45.4	9.6	5.4	12.6
WSU Administration	8.2	23.1	15.4	5.4	47.8

PART II. WSU WHEAT BREEDING PROGRAMS

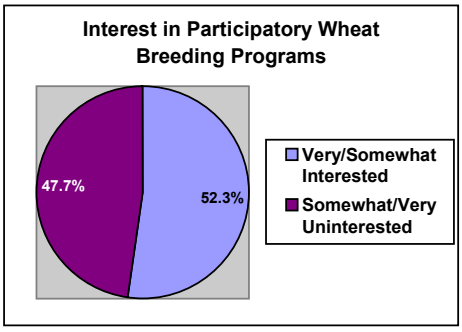
Growers' satisfaction with WSU's winter and spring wheat breeding programs.



Growers' perceptions of how much priority WSU's wheat breeding programs should give to the following wheat characteristics.

	High priority (%)	Medium priority (%)	Low priority (%)
Wheat requiring less nitrogen for given yield and protein content	68.3	30.0	1.7
Wheat with greater genetic diversity for pest and disease control	65.5	31.3	3.2
Specialized wheat for market segments	55.3	36.5	8.3
Herbicide tolerant wheat	45.5	44.0	10.5
Wheat suited for marginal production areas	37.7	35.6	26.7
Wheat with non-food uses (industrial, energy)	30.2	43.3	26.5
Perennial wheat	29.0	41.2	29.2

Growers' interest in working directly with WSU scientists in participatory wheat breeding programs within the next 1 – 3 years.



Participatory Wheat Breeding uses both breeder and farmer expertise to develop varieties particularly suited to a specific set of environmental challenges.

Growers' familiarity with WSU's effort to breed perennial wheat.

	Very familiar (%)	Somewhat familiar (%)	Somewhat unfamiliar (%)	Not familiar (%)
Familiarity with WSU's perennial wheat breeding effort	6.2	41.0	23.2	29.6

Growers' interest in planting perennial wheat with the following characteristics.

	Very interested (%)	Somewhat interested (%)	Somewhat uninterested (%)	Very uninterested (%)
Mixes well with annual wheat, using the same equipment	52.3	33.2	8.6	5.9
Meets minimum yield requirement	48.8	35.4	8.6	7.2
Grows well in highly erodible areas	48.3	35.0	9.6	7.1
Works well under specific conservation programs or regulations	35.8	45.7	11.7	6.7
Suited for currently unproductive parts of fields	35.5	33.9	20.3	10.4

Percentage of growers who planted private and public wheat varieties during 2003 – 2005.

	2003 (%)	2004 (%)	2005 (%)
Private wheat varieties	40.4	43.8	44.2
Public wheat varieties	94.0	94.8	94.8

Percentage of growers who saved private and public wheat varieties during 2003 – 2005.

	2003 (%)	2004 (%)	2005 (%)
Private wheat varieties	9.4	8.8	7.8
Public wheat varieties	24.7	25.6	24.9

Growers' frequency of planting wheat seed saved from their own fields.

	Every year (%)	Every other year (%)	Sometimes but less than every other year (%)	Not at all (%)
Frequency of planting saved wheat seed	20.0	5.1	13.4	61.5

Growers' perception of the importance of various factors when deciding whether or not to save their wheat seed.

	Extremely important (%)	Mostly important (%)	Slightly important (%)	Not important (%)
Knowledge necessary to ensure quality	42.4	29.8	12.9	14.9
Extra storage capability	30.8	30.6	18.7	19.8
Time/management	30.3	36.0	17.4	16.3
Availability of necessary machinery	28.9	33.9	18.2	19.1

Percentage of growers who save seed for crops other than wheat.

	Yes (%)	No (%)
Save seed for crops (other than wheat)	19.7*	80.3

* Seeds saved include barley, oats, lentils, peas, triticale and garbanzos.

PART III. SUCCESSFUL WHEAT PRODUCTION AND MARKETING STRATEGIES

Growers' perceptions of the importance of various factors in their efforts to make their wheat farm operations more successful.

	Extremely important (%)	Mostly important (%)	Slightly important (%)	Not important (%)
Ensuring high yields	87.3	11.7	1.0	0.0
Lowering input costs	82.6	15.9	1.5	0.0
Increasing the number of buyers and markets for wheat	80.5	15.1	4.0	0.4
Developing alternative uses for wheat (e.g., bioenergy, industrial products)	56.5	30.0	11.9	1.5
Preventing pest resistance	51.2	42.6	5.6	0.6
Rebuilding regional storage and transportation networks	32.1	35.0	27.6	5.3
Increasing uniformity in the field	31.4	44.8	22.4	1.4
Promoting genetic diversity in wheat varieties	27.6	50.4	20.0	2.0
Emphasizing environmental conservation	26.2	45.4	25.4	3.0

Growers' opinions about the future of wheat production in Eastern Washington.

	Strongly agree (%)	Somewhat agree (%)	Somewhat disagree (%)	Strongly disagree (%)
Specific wheat varieties should be grown only in appropriate geographic areas dues to quality concerns	26.8	57.8	13.1	2.3
All wheat varieties should meet minimum quality standards for seed to be sold in the state	61.5	31.3	6.5	0.7
Old wheat varieties should be taken off the market when new ones replace them	3.8	31.6	47.1	17.5
University plant breeding programs are a necessary component of a sustainable farm economy	79.5	19.4	0.9	0.2
Government supported agricultural programs should be targeted to benefit small and medium sized farms	42.8	34.8	16.5	5.9
Publicly funded agricultural research and extension should be expanded	49.5	45.6	4.5	0.4
Research and consultation by private agribusiness firms can replace most of the work done by university research and extension	1.1	14.2	48.3	36.4

Growers' interest in various types of wheat marketing strategies.

	Extremely interested (%)	Mostly interested (%)	Slightly interested (%)	Not interested (%)
Increased emphasis on delivering high quality clean wheat to domestic and overseas buyers, with premiums for growers who deliver above standards	68.1	26.6	4.3	0.9
Niche marketing of high-value wheat varieties or products	32.8	40.0	23.0	4.2
Rebuilding regional infrastructure for more local control of processing, distribution and marketing	24.2	47.9	24.0	3.8
Marketing club that pools specific varieties to sell directly to end users	21.5	44.7	27.9	5.9
Maintain current commodity system	16.2	51.7	27.5	4.6

Growers' perceptions of how much the following challenges negatively affected their farm operations during 2003 – 2005.

	Highly affected (%)	Somewhat affected (%)	Hardly affected (%)	Not affected (%)
Low commodity prices	93.7	4.6	1.1	0.6
High input costs	88.9	9.8	0.8	0.6
Limited market opportunities	47.9	39.6	9.5	3.0
Federal agricultural policy and regulations	34.7	45.8	14.3	5.2
Too few companies buying commodities	32.1	45.7	17.2	4.9
Declining number of family farms	18.8	30.6	29.8	20.8
Declining population in small towns	15.6	27.3	31.6	25.6
Amount of land in CRP	9.7	22.8	34.3	33.2
WSU research not focused on farmer needs	9.0	37.7	35.1	18.2
Too few machinery dealers in my area	4.5	28.6	36.2	30.7
Too few input suppliers in my area	4.2	23.7	37.0	35.1
Access to loans	3.4	14.7	34.7	47.2

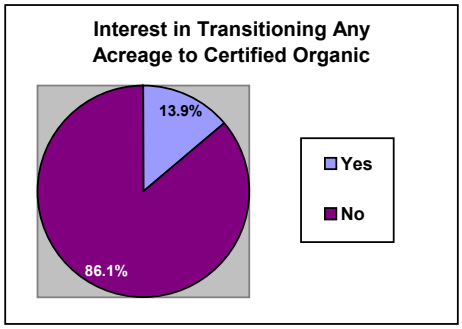
PART IV. ORGANIC FARMING

Only three (3) growers reported that parts of their farms were certified organic. The survey asked all of the remaining growers the following two questions:

Which of the following are your main reasons for NOT having any certified organic acres? *Circle all that apply.*

	Percentage of growers who circled each reason (%)
Organic weed control methods are inadequate	69.3
Cannot get same yields with organic as conventional methods	59.3
Organic pest/disease control methods are inadequate	58.9
Not worth the time	43.4
Transportation and access to organic buyers are limited	36.3
Too difficult to get enough nitrogen	35.9
Need more information on organic production	33.2
Certification is too much trouble	24.3
Other reasons	17.8

Within the last five years, 2001 – 2005, have you considered transitioning any of your acreage to certified organic?



PART V. GENETICALLY MODIFIED WHEAT

Growers' perceptions of the extent to which the requirement that growers sign "Technology Use Agreements" (that prohibit the saving and replanting of seed) would influence their decision about planting genetically modified crops.

	Very high influence (%)	Somewhat high influence (%)	Moderate influence (%)	Somewhat low influence (%)	Very low to no influence (%)
Extent of influence	12.5	12.7	18.2	15.0	41.6

Percentage of growers who agree/disagree with the decision by a private firm to suspend a request for government approval for the sale of Roundup Ready® wheat seed.

Agree	47.4%

Of those who agreed...

VIEWS ON RR® WHEAT SEED	%
Hope that non-GM herbicide tolerant wheat will be developed in the future	29.9
Hope that approval for Roundup Ready® wheat seed will be sought in future	27.1
Hope that approval for Roundup Ready® wheat seed will not be sought in the future	14.0
Other viewpoints	29.0

Disagree	52.6%

Of those who disagreed…

VIEWS ON RR® WHEAT SEED	%
Roundup Ready® wheat seed is a technology that farmers currently need	70.7
A non-GM herbicide tolerant wheat is needed in the near future.	18.1
Roundup Ready® wheat seed is unnecessary technology	3.2
Other viewpoints	8.0

Percentage of growers who would consider planting genetically modified wheat varieties on their farms.

Yes	65.8%

Of those who would consider…

REASONS FOR CONSIDERING	%
GM wheat has traits that would reduce input costs	49.2
GM wheat would make management easier	22.7
GM wheat has specific end-user traits that would result in a price premium	15.3
Other reasons	12.8

No	34.2%

Of those who would not consider…

REASONS FOR NOT CONSIDERING	%
Too many uncertainties in the market	63.6
Too many issues with liability and patent ownership	10.3
Too many uncertainties about the environmental effects	7.9
Other reasons	18.2

PART VI. GENERAL BACKGROUND INFORMATION

Average size (in terms of acres) of growers' farm/wheat operations.

	Average across all growers
Acres currently farmed	3,145 acres
Acres of winter wheat (2005)	1,183 acres
Acres of spring wheat (2005)	280 acres

Growers' perceptions of the importance of various information sources on their decision-making about issues related to growing wheat.

	Extremely important (%)	Mostly important (%)	Slightly important (%)	Not important (%)
Agricultural input supplier consultations	36.4	46.0	13.2	4.3
WSU research program field days	33.5	39.6	20.4	6.5
Spouse or business partner	31.4	29.6	20.4	18.6
Washington Association of Wheat Growers mailings	24.9	45.7	25.4	4.0
WSU Extension agent or scientist	21.8	43.0	28.4	6.8
WSU Extension bulletin	20.8	48.6	25.4	5.2
Washington Association of Wheat Growers meetings	12.8	27.2	42.5	17.5
Relatives	11.2	27.0	30.3	31.5
Agricultural input supplier magazines	10.5	34.2	42.7	12.7
Neighbors	10.1	35.6	36.6	17.8
Farm Bureau mailings	6.3	19.0	27.4	47.2
Farm Bureau meetings	2.5	11.0	32.7	53.8

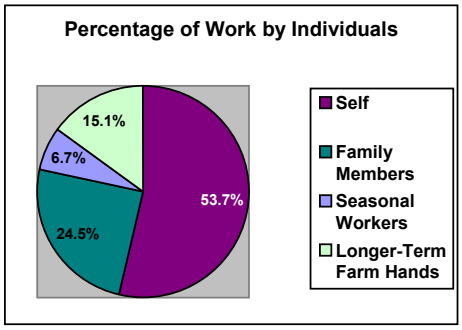
Total number of field days attended during 2001 – 2005 (average across all growers).

	Average across all growers
Number of field days attended	3.0 field days

Percentage of growers with different types of farm business organizations.

	Percentage of growers (%)
Family corporation	43.7
Single family or individual operation	29.1
Family partnership	22.1
Cooperative, estate, or trust	1.4
Non-family partnership	1.0
Non-family corporation	0.8
Other	2.0

Percentage of work supplied by different individuals on growers' farms.



Percentage of income from off-farm employment (average across all growers).

	Average across all growers (%)
Percentage of income from off-farm employment	22.0

Estimated cost of production per acre in 2005 (including costs of seed, fuel, fertilizer, pest and weed control, equipment, taxes, labor, etc.).

	Average across all growers
Estimated cost of production per acre	$152

Percentage of growers who reported the following total farm receipts for their farm businesses in 2005.

	Percentage of growers (%)
Less than $2,500 in total farm receipts	0.2
$2,500 to $24,999 in total farm receipts	4.4
$25,000 to $49,999 in total farm receipts	4.0
$50,000 to $99,999 in total farm receipts	7.7
$100,000 to $249,999 in total farm receipts	35.9
$250,000 to $499,999 in total farm receipts	30.5
$500,000 or more in total farm receipts	17.3

Percentage of growers who reported the following percentage ranges for their total household income derived from the farm operation.

	Percentage of growers (%)
0 – 24% of household income from farming	14.4
25 – 49% of household income from farming	13.4
50 – 74% of household income from farming	19.6
75 – 100% of household income from farming	52.7

Highest level of education completed by growers who responded to survey.

	Percentage of growers (%)
Less than high school degree	1.4
High school degree	8.5
Some college	25.8
Vocational degree	10.0
College degree	40.6
Some postgraduate work	5.9
Postgraduate degree	7.9

Photo Credits: Title Page: Jim Shroyer, Kansas State University; Pages 2 and 12: WSU Winter Wheat; Page 5: WSU Marketing and Communications Department; Page 7: Jessica Goldberger, WSU