IDENTIFICATION OF SOURCES OF RESISTANCE TO RHIZOCTONIA ROOT ROT IN MUTANT AND WILD BARLEY

By

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To the Faulty of Washington State University:

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Abstract

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Rhizoctonia root rot and bare-patch caused by *Rhizoctonia solani* AG-8 is an important disease that limits yields of cereal crops in no-till/direct seeding systems in the Pacific Northwest (PNW) region of the United States, and in turn limiting the widespread adoption of this system in this region. Due to the dearth of commercial cereal cultivars that are genetically resistant to the fungus, growers have mostly relied on the combination of various cultural practices to combat the disease. Unfortunately these practices do not eliminate the pathogen from the soil or significantly increase yield. Spring barley is highly susceptible to the disease. However, spring barley serves as an important rotational crop in the PNW offering growers with several rotation benefits such as disease reduction and increased productivity, especially when grown in rotation with wheat. With the objective of identifying sources of resistance to the disease in barley, two different experiments were conducted. In the first experiment, M_2 populations of sodium azide barley mutants of cultivar Lenetah and breeding line 05WA-316.99 were screened for disease reaction to the fungus. Putative mutants were verified to confirm observed resistance. From these two lines, 10 putative M_3 individuals were identified and crossed to wild-type progenitor for genetic analysis. Evaluating the BC_1F_1 plants of verified putative mutants for disease reaction, a greater

number of susceptible plants were observed based on disease severity ratings and other parameters measured. These findings suggest that resistance to Rhizoctonia root rot in mutant barley is inherited as a dominant trait. Given the importance of wild crop relatives to crop improvement, the second experiment involved exploring the possibility of identifying resistance to the disease in the direct progenitor of cultivated barley *Hordeum vulgare* subsp. *spontaneum*. Of the entire pool of 317 accessions in the Wild Barley Diversity Collection that was screened for resistance, six accessions showed potential as gene donors for *Rhizoctonia* resistance with one accession, WBDC 021 showing the greatest potential by displaying moderate resistance to the pathogen.

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CHAPTER ONE

INTRODUCTION

The Pacific Northwest (PNW) of the United States has a favorable environment for the production of cool season grasses including wheat and barley because of the soil and climatic conditions (Cook, 1992; Schroeder and Paulitz, 2006). Traditional farming practices in this region have, over the years, involved tillage-intensive wheat-based cropping systems. The low rainfall regions of the PNW receives less that 300 mm of average annual precipitation, and constitutes the largest cropping zone in western United States (Schillinger et al, 2006). In this zone, the trend is to produce winter wheat in a 2-yr rotation with summer fallow. In the high precipitation regions (receiving between 300- 600 mm of average annual precipitation), winter wheat is grown in rotation with spring crops such as barley, peas, lentils, canola and chickpeas (Smiley et al., 1993; Schillinger et al., 2006). At least two-thirds of all rotations in the PNW involve wheat and barley (Cook, 2001) with spring barley being an important annual crop in 2 or 3-yr rotations with winter wheat, peas, lentils or fallow (Smiley et al., 1992).

Seed bed preparation traditionally involves intensive tillage operations during the fallow period to bury the previous crop's residue, break up soil clods and provide a more uniform soil surface for planting (Schillinger and Papendick, 1997). Soils in this region are of a silt loam texture, and in the low precipitation areas, are prone to wind erosion because of limited vegetation on soil surface, dry environmental conditions and high winds, while water erosion is the major cause of soil loss in the high precipitation areas (Papendick, 2004; Schillinger et al., 2006). These soils are reported to have lost about 50% of their soil organic carbon (SOC) as a result of topsoil erosion and oxidation (Kennedy, 2004).

Intensive tillage operations that are carried out prior to planting result in significant loss of topsoil which constitutes major agronomic and environmental concerns for growers in this region. For example, in the low precipitation regions, dust particles arising from high winds in intensively tilled fields contribute to loss of topsoil and also reduce air quality. In the high rainfall regions where water erosion is more of a problem, dune-like hills with steep slopes, rainfall on thawed soils covering a frozen layer (McCool, 1990) coupled with intensive tillage operations contribute to significant soil loss (Schillinger et al., 2006).

Considering the erosion problems associated with intensive tillage operations in all precipitation zones, growers are becoming increasingly interested in no-till technology in order to improve soil quality through a reduction in wind and water erosion and at the same time, reduce costs of inputs since most tillage operations would be eliminated under no-till conditions. No-till or direct seeding is the practice of sowing directly into the stubble or residue of the previous crop without any form of tillage activity that stirs or turns the soil before planting (Cook et al., 2002). No-till offers growers several agronomic, economic and environmental benefits including significant savings in production costs, reduced erosion problems (Papendick et al., 2004) due to ample residue cover on soil surface, improved water infiltration and storage, and increased soil organic carbon (Nyakatawa et al., 2001). However, with the adoption of reduced or no tillage coupled with an increased frequency of cereal crops in rotation in the PNW, especially barley-wheat rotations, root diseases have become major problems to manage (Cook, 2001). These diseases limit the full adoption of no-till practices in this region (Paulitz et al., 2002). Several fungal root diseases decrease the yield and quality of wheat and barley produced in the PNW, including take-all (*Gaeumannomyces graminis* (Sacc.) Arx & D. Oliver var. *tritici* J. Walker), Rhizoctonia root rot

(*Rhizoctonia solani* Kuhn and *R. oryzae* Ryker and Gooch) and Pythium (*Pythium* Pringsh. spp.) root rot (Schroeder and Paulitz, 2006). The most important especially for spring barley is Rhizoctonia root rot caused by *Rhizoctonia solani* AG-8 and *Rhizoctonia oryzae*, which is one of the major factors limiting crop yield in no-till systems (Pumphrey et al., 1987; Weller et al., 1986; Ogoshi et al., 1990; Cook, 2001; Paulitz et al., 2002). Also, when compared to wheat, spring barley is more affected and shows more stunting than wheat at similar inoculum levels (Paulitz and Steffenson, 2011).

Elimination of the 'green bridge' by getting rid of volunteer crop plants and grassy weeds using a pre-plant herbicide 2-3 weeks before planting is known to decrease disease incidence to low levels (Smiley et al., 1992). At the same time, ensuring some form of soil disturbance in the seed zone during planting is also known to reduce disease severity (Roget et al., 1996). However, other management options including the use of fungicides and the application of starter fertilizers beside the seed have not proven effective in controlling the disease (Paulitz et al., 2002). Crop rotation does not offer full protection either due to the wide host range of *Rhizoctonia solani* (Cook et al., 2002). At present, there is no wheat or barley genotype that is known to be genetically resistant to Rhizoctonia root rot (Smith et al., 2003). If widespread adoption of direct seeding systems is to be achieved in the PNW, genotypes that are able to adapt to no-till conditions would need to be identified or developed. The use of resistant barley cultivars in the normal 2-3 year rotations with winter wheat under reduced tillage in any growing environment would help reduce the inoculum levels of this pathogen in the soil. At the same time, barley lines that are resistant to Rhizoctonia root rot could serve as potential sources of resistant genes for use in wheat improvement, as well.

Therefore, the objectives of this study are:

1) To identify potential sources of genetic resistance to Rhizoctonia root rot in barley by screening mutagenized *Hordeum* germplasm/populations and wild barley accessions for disease response to isolates of *Rhizoctonia solani* AG-8.

2) To study and determine the inheritance of resistance in the progeny of verified mutants

LITERATURE REVIEW

Dryland Cropping in the Inland Pacific Northwest

The Inland Pacific Northwest (PNW) which includes eastern and central Washington, northern Idaho and north central Oregon is one of the main regions recognized for dryland agriculture in the western USA (Schillinger et al., 2006). Although both dryland and irrigated agriculture are practiced in the PNW, dryland farming occurs on about 3.3 million ha while the land area under irrigated agriculture is estimated to be about 0.6 million ha (Schillinger et al., 2006). Dryland agriculture has been defined as one which receives 600 mm or less average annual precipitation without irrigation (Schillinger et al., 2006). The PNW is dominated by a Mediterranean–like climate having cool to cold winters and summers with warm and dry conditions. Most of the precipitation received in these regions occur between November and April, with 20% of the total precipitation occurring at higher latitudes and altitudes in the form of snow (Papendick, 1996). Average annual precipitation as low as 150 mm, occurs in south-central Washington and increases from west to east to about 600 mm in the Palouse region (Schillinger et al., 2010). Although rainfall intensities of the region are generally low (Papendick et al., 1996), the climate, soils, topography and the farming systems are major factors responsible for the runoff and

erosion losses that occur in this region (Naffziger and Horner, 1958). The dryland regions of the PNW are divided into three zones on the basis of average annual precipitation. These include the low, intermediate and high precipitation zones receiving <300, 300-450 and 450-600 mm of average annual precipitation respectively (Schillinger et al., 2010). The low precipitation zone, receiving an average annual precipitation of between 150 mm to 300 mm, comprises about 1.56 million ha of land under dryland agriculture (Schillinger et al., 2006). The zone is characterized by gently rolling topography and the soils are a product of windblown silt deposits called loess which came about as a result of the glacial lake Missoula flooding that occurred some 15,000 years ago (Busacca, 1991). Different soil types ranging from organic matter-rich Mollisols to low organic matter Aridisols as well as Entisols are found to occur in this zone (Boling et al., 1998). Each soil type is specific to certain regions depending on the amount of average annual precipitation received. Soil texture ranges from sandy loams to silt loams with the former occurring more in areas that receive very little precipitation. Soils are permeable with good drainage, and in some areas, are of sufficient depth to allow storage of winter precipitation (Papendick, 1996). According to Schillinger et al (2006), many years of intensive farming in this moisture zone have resulted in soils that have lost about 50% of organic matter content to erosion and oxidation. Given the moisture limitations and other environmental conditions, the traditional cropping system adopted over the years is the tillage-based, 2-year winter wheat-summer fallow (WW-SF) in which one crop of winter wheat is sown every two years (Cook et al., 2002; Schillinger et al., 2010). The fallow period is necessary to ensure the accumulation and storage of 'overwinter' precipitation to help stabilize subsequent crop yields and lower the risk of crop failure that may occur due to drought (Ramig, 1983; Cook et al., 2002). During the summer fallow period, growers

traditionally carry out a minimum of eight tillage operations to bury soil surface residues, kill weeds, break up soil clods and improve soil surface smoothness (Schillinger and Papendick, 1997). Dust particles produced from intensively tilled fields by blowing winds contribute to significant soil loss and air quality problems (McCool, 1990). As a result, growers make use of herbicides (chemical fallow) to control weeds. Although the use of chemical fallow reduces the amount of soil lost as a result of wind erosion and also improves air quality, the adoption rate is very low due to limited amount of seed-zone moisture available for crop establishment in the fall and hence the lower yields observed in chemical fallowed soils compared to soils that have been tilled in the summer -fallow period (Schillinger and Bolton, 1993; Papendick, 1996).

Although between 60-75% of the total precipitation that occurs during the fallow period is stored in the soil, only about 30% of precipitation received in the fallow year is utilized by wheat plants sown in the succeeding cropping year as most of the moisture is lost to runoff and evaporation (Leggett et al., 1974; Cook et al., 2002). The WW-SF system is currently practiced on about 90% of the cropland under cultivation with average grain yields ranging from 1200 to 3700 kg ha^{-1} (Schillinger et al., 2006). The relatively stable grain yields and less risk associated with the WW-SF rotation makes it the preferred cropping practice although other cropping systems such as a 3-year winter wheat-spring cereal-fallow rotation and the continuous annual cropping also occur (Cook et al., 2002; Schillinger et al., 2006). The 3-year winter wheat-spring cereal-fallow rotation is practiced on about 10% of croplands when sufficient amount of overwinter precipitation is stored in the soil up to a depth of 1 m (Cook et al., 2002). Although yields of spring crops in the 3-year winter-wheat-spring cereal fallow rotation are highly variable ranging from 700 to 2,500 kg ha^{-1} , the rotation system have been able to compete agronomically and economically with the WW-SF cropping system

(Schillinger et al., 2010). But continuous annual cropping is practiced on less than 1% of croplands (Cook et al., 2002). In the low precipitation zone, drought, coupled with conditions of inadequate crop residue, high winds, excessive tillage and low organic matter contribute to the vulnerability of the soils in the region to the erosive action of wind (Papendick, 2004).

The high and intermediate precipitation zones are characterized by dune-like hills with steep slopes that make these areas more prone to water erosion. Both primary and secondary tillage operations in the intermediate zones involve chisel plowing, disking and about two or three cultivator and harrow operations (Papendick, 1996). Primary tillage operations in the high precipitation zone previously involved the sole use of moldboard plow, however, the use of the chisel plough for primary tillage and field cultivator, rod weeder and harrow for secondary tillage is the more recent practice (Papendick, 1996; Schillinger et al., 2003).

Due to the negative impact of intensive tillage operations on soils in all precipitation zones, growers have been experimenting and sometimes adopting more sustainable management options that can help improve soil, quality reduce rates of soil loss and enhance the yield potential of the land. Growers in the PNW have been shifting towards adopting reduced-till and no-till/ direct seeding. Reduced tillage systems use alternatives to the moldboard plow such as disk and chisel plow or harrows. These implements leave a less rough and higher residue surface. No-till or direct seeding is a practice of sowing directly into the residue to previous crop without any form of soil disturbance (Cook et al., 2002). In no-till, both planting and fertilizer application are done in one pass (Cook, 2001) and the only form of soil disturbance is that which is needed to plant seed and inject fertilizer (Schroeder and Paulitz, 2008).

No-till offers growers with several environmental, agronomic and economic

benefits. Maintaining crop residue on the soil surface protects the soil from water and wind erosion (Papendick, 2004), increases soil organic carbon build up (Nyakatawa et al., 2001) thereby improving soil quality (Doran et al., 1996), increases the population and diversity of soil microorganisms, and also increases the soil organic matter levels (Stubbs et al., 2004). Tillage may also improve infiltration and storage of water in the soil since crop residue helps to hold more moisture (Paulitz et al., 2002). It is also believed that no-till or reduced tillage has the ability to sequester carbon thereby reducing the amount of greenhouse gas emitted into the atmosphere as CO_2 (Stubbs et al., 2004). Finally, no-till results in reduced fuel and labor costs since many tillage operations have been eliminated and there are only few passes over the field (Cook et al., 2002).

Despite the numerous benefits of reduced tillage or no-till, the rate of adoption by growers in the PNW is still very low for example, the low precipitation regions of the PNW make up the largest cropping zone in western USA (Schillinger et al., 2006) and no-till is still reported to be practised on only about 5% of cropland in western USA (CTIC, 2001). Aside from weed management problems and transition costs, one of the major reasons identified for the low rate of transition to no-till by growers in the PNW is the incidence of soilborne diseases associated with no-till or reduced tillage (Paulitz, 2006). The shift towards no-till technology in a cereal based growing environment like the PNW has been identified as one of the reasons for an increased occurrence of soilborne root diseases (Cook, 2001). Rhizoctonia root rot (*Rhizoctonia solani* AG-8) is an important disease in direct seeding (Weller et al., 1986; Smiley and Wilkins, 1993) systems and in the first few years of transition from conventional tillage to no-ill (Schroeder and Paulitz, 2006). The disease has been identified as the most important

disease of spring barley that is sown directly into cereal stubble under the PNW growing conditions (Pumphrey et al., 1987; Weller et al., 1986; Ogoshi et al., 1990).

Rhizoctonia root rot and bare patch disease

The French mycologist, A. P. De Candolle in the year 1815 was the first to describe the genus *Rhizoctonia* (translated 'death of plants' in Greek) for the violet root rot organism that he observed to ''rapidly attack and kill the roots of phanerogamic plants'' (Menzies, 1970). Several years later (1858), a certain fungus observed on diseased potato tubers was named *Rhizoctonia solani* by the German agronomist, Julius Kühn (Kühn, 1858) and has since its identification become one of the well-known devastating plant pathogen affecting crop plants (Menzies, 1970).

Rhizoctonia solani Kühn [teleomorph: *Thanatephorus cucumeris* (Frank) Donk], is the most studied species in the genus *Rhizoctonia* (Sneh et al., 1991). The genus includes a ubiquitous group of fungi that are naturally occurring in both cultivated (Ogoshi, 1987) and uncultivated areas of different parts of the world causing diseases symptoms such as damping off, root rots, seed decay, fruit decay, stem cankers and foliage diseases on a wide range of host plants (Menzies, 1970).

Isolates of *R. solani* are assembled into anastomosis groups (AG) based on affinities for hyphal fusion by different isolates paired on a medium such as water agar. Isolates belonging to the same anastomosis group show hyphal fusion and death of fused cells (Yokoyama and Ogoshi, 1984). According to Sneh et al. (1991), *R. solani* isolates are presently subdivided into eleven anastomosis groups. AG-8 isolates have been reported as important cereal root pathogens (Neate and Warcup, 1985; Roberts and Sivasithamparam, 1986; Rovira et al., 1986).

Rhizoctonia root rot and bare patch caused by *R. solani* is a major disease in direct seeded or no till cereals in Australia (MacNish et al., 1996) and the Pacific Northwest region of the United States (Weller et al. 1986). The disease was first described in Australia in the late 1920s (Samuel, 1928) where it was initially referred to as 'stunting disease' (Samuel, 1928) and later on as purple patch (Hynes, 1933) and bare batch (Ludbrook et al., 1953). In 1984, the disease was observed in the Pacific Northwest where it occurred as patches of stunted plants in direct seeded wheat and barley fields of Washington, Idaho and Oregon (Weller et al., 1986). It has also been reported in England (Dillon-Weston et al., 1943) and Scotland (McKelvie, 1978; Murray, 1981).

Initial attempts at discovering which anastomosis group of *Rhizoctonia solani* was responsible for the Rhizoctonia root rot and bare patch revealed AG-8 as the major strain (Neate and Warcup, 1985). AG-8 was found to be associated with diseased plants within Rhizoctonia bare patches and was highly pathogenic to roots of cereals and other crops (Neate and Warcup, 1985; MacNish and Sweetingham, 1993).

However, in the Pacific Northwest, the most abundant and virulent species of Rhizoctonia in infected fields include *R. solani* Kühn AG-8 (Neate and Warcup, 1985) (Telemorph *Thanatephorus cucumeris* Frank Donk) and *R. oryzae* Ryker and Gooch (Ogoshi, et al., 1990) (Telemorph *Waitea circinata* Warcup and Talbot WAG-0). Both species of *Rhizoctonia* have been found to be potentially responsible for the observed root rot and stunting in wheat and barley fields of this region (Ogoshi et al., 1990). *R. solani* AG-8 isolates were found to be highly pathogenic at lower temperatures of 10°C with plants showing mild disease symptoms as temperatures increased. However, *R. oryzae* was highly pathogenic at 20°C with minimal disease symptoms at lower temperatures (Ogoshi et al., 1990).

In Australia where the disease was first described, it occurred in patches of 3-6 feet in diameter. Affected plants exhibited stunted growth, reduced tillering and stiff, rolled leaves. Moreover, the most distinguishing symptom of the disease was mostly observed on the roots of affected plants. Root tips of diseased plants appeared severely damaged or killed thereby stimulating the growth of lateral roots, which also became severely affected. Similar and additional symptoms have been observed by other authors. Bare patches and root rot of plants outside bare patch are the common symptoms on cereals (Hynes, 1937) with bare patch being the most striking form of the disease attracting much attention and interest. According to MacNish and Neate (1996), the bare patch symptom is characterized by patches of stunted plants and subsequent death of many plants within patches. In most cases, stunted plants within patch areas show a decline in growth during the growing season. Although many of these plants recover if the extent of stunting is not severe, such plants produce fewer tillers and give poorer yields (MacNish and Neate, 1996). Patches in the field become noticeable from 3-4 weeks after sowing (Weller et al., 1986). These patches appear circular but mostly elongated in the direction of sowing (MacNish, 1985; Robert and Sivasithamparam, 1986) and are usually clustered to form larger patches (MacNish, 1985). Plants located within patches exhibit several root rot symptoms (Rovira, 1986; Robert and Sivasithamparam, 1986).

Roots of infected plants exhibit characteristic brownish lesions with discolored sharp pointed and/or pruned tips referred to as ‘‘spear tips’’ (MacNish and Fang, 1987). In addition to root rot symptoms, plants within patches also display symptoms characteristic of moisture and nutrition stress (MacNish and Neate, 1996), rolled leaf blades, chlorosis of lower leaves and tillering inabilities (Hynes, 1937).

Considering the effects of soil type on the severity of the disease, Rhizoctonia bare patch was initially observed in 'sandy calcareous' soils of South Australia (Samuel and Garrett, 1932). MacNish and Neate (1996) however reported that the severity of root rot was the same for all soil types in Australia while bare patch conditions increased as the soils become lighter.

The conventional farming practice in the drier areas of the Pacific Northwest is the winter wheat-summer fallow rotation allowing for only one crop to be sown every two years. The major tillage operations performed in the summer fallow include burying of surface residues and breaking of soil clods to reduce surface roughness (Schillinger and Papendick, 1997). However, in order to conserve moisture, lower cost inputs, reduce erosion, and improve soil organic matter, farmers in the Pacific Northwest are developing increasing interest in the adoption of no till or direct seeding. Cook et al. (2002) defined direct seeding as ''fertilizing and planting directly into the residue of the previous crop without any tillage operations that mix or stir the soil prior to fertilizing and planting''. However, it has been found that pathogens responsible for root diseases in wheat and barley plants thrive longer in soils that have been undisturbed and covered with crop residue (Cook, 1992). Also, several studies have shown the association of Rhizoctonia root rot with no-till or direct seeding systems (Weller et al., 1986; Pumphrey et al., 1987; Smiley and Wilkins 1993; Roget et al., 1996) and in the early years of transition from conventional tillage to direct seeding (Schroeder and Paulitz, 2006). Therefore, an increase of Rhizoctonia bare patch in no till systems (Rovira, 1986; Pumphrey et al., 1987) has been a major hindrance to the adoption of no till in the Pacific Northwest (Schroeder and Paulitz, 2006).

Management of Rhizoctonia root rot and bare patch.

There is presently no single method of controlling Rhizoctonia root rot and bare patch, however, various combinations of available management methods can achieve desired results (MacNish and Neate, 1996). Various field experiments have demonstrated the importance of cultivation (tillage) in reducing the severity of the disease (Pumphrey et al., 1987; MacNish, 1985; Jarvis and Brennan, 1986), however, it does not completely eliminate the pathogen (MacNish, 1985). The mechanism by which cultivation reduces the disease is unknown (MacNish and Neate, 1996) although, several possible mechanisms have been suggested. For example, because plant debris serves as source of Rhizoctonia inoculum (Neate, 1987), cultivation may either prevent an increase in organic matter/plant debris accumulation (Rovira et al., 1985) or serve to break up the contacts between the hyphae of the pathogen present on plant residue (McDonald et al., 1985; Gill et al., 2002; Paulitz et al., 2002). Another explanation for how tillage might reduce the severity of the disease might be due to the production of abundant suppressive microbes that are associated with the incorporation and decomposition of plant debris during tillage activities (Paulitz et al., 2002).

The severity of Rhizoctonia root rot in no-till systems has been attributed to the presence of volunteer crops or grassy weeds that remain on the field between harvest and subsequent planting. This condition known as ‘green bridge’ has been found to favor an increased inoculum potential of many plant pathogens especially *R. solani* (Smiley et al., 1992). Sowing wheat or barley seeds directly into the stubble of volunteers and weeds that have been sprayed 2-3 days earlier with pre-planting herbicides like glyphosate increases the severity of the disease (Roget et al., 1987; Pumphrey et al., 1987). The application of glyphosate to kill weeds results in a breakdown of the defense mechanism of plants against pathogens by blocking the

shikimic acid pathway (Levesque and Rahe, 1992). Due to a defense breakdown, dying weeds and volunteer crops become colonized by Rhizoctonia inoculum and serve as bridging hosts that amplify the amount of inoculum present. Wheat or barley seed that are sown directly into treated weeds and volunteers a few days after herbicide application emerge at a time when the inoculum density is high and therefore become strongly affected by these high inoculums levels. Elimination of the 'green bridge' has been found to address this problem (Roget et al., 1987; Smiley et al., 1992). According to Roget et al (1987), allowing a period of 3-6 weeks between the time of herbicide application and sowing reduced disease severity, risk of damage from the disease and resulted in an 83% increase in yield of direct seeded wheat. Smiley et al. (1992) reported that contrary to planting 2-3 days after spraying, a prolonged interval of about 3 weeks from time of herbicide spray to planting reduced severity of root rot and increased yield of spring barley. More recent field experiments observed a reduction in disease severity on barley plants with an interval of 11-27 days and 13-37 days respectively between time of herbicide application and planting (Babiker et al., 2011).

In addition to the elimination of green bridge, seed treatment as a management technique for the disease found a 37% grain yield increase for spring barley in infested fields when seeds were treated with a mixture of carboxin, thiram, iprodione and chloroneb (Cook, 1986). However, subsequent fungicide seed treatments were found ineffective for managing the disease on two winter wheat cultivars in Eastern Oregon (Smiley et al., 1990).

Nitrogen fertilizer applied as urea, sodium nitrate or ammonium sulphate was shown to reduce the root rot and bare patch disease (MacNish, 1985); however, the effect of nitrogen fertilizer in reducing the disease was found to be inconsistent in subsequent trials (MacNish, 1988). Similar results were also obtained by Pumphrey et

al. (1987) in a field experiment set up to study the effect of tillage and nitrogen fertilizer on the disease in which both the amount and timing of fertilizer application did not impact the frequency of patches.

Control of the Rhizoctonia bare patch and root rot disease by crop rotation has met with little success due to the wide host range (Rovira, 1986) which has been recorded with wheat (*Triticum aestivum* L.), oats (*Avena sativa* L.) (Samuel, 1928), barley (*Hordeum vulgare* L.) (Samuel and Garrett, 1932), cereal rye (Hynes, 1933), rapeseed (*Brassica napus* L.) (MacNish, 1983), annual medics (*Medicago* spp), lucerne (*Medicago sativa* L.) and subterranean clover (*Trifolium subterraneum* L.) (Ludbrook, et al., 1953). Inclusion of broadleaf crops in rotation has also been shown to offer no benefit for the control of Rhizoctonia root disease as most broadleaf crops that are available for rotation with cereals are found to be susceptible to *R. solani* AG-8 (Cook et al., 2002). Studies on crop rotation as a management strategy to control the disease have reported that patch areas in wheat planted after a mixed pasture of grass and medic were found to be greater than in wheat that followed medic pasture, peas or wheat (Rovira, 1986). Schillinger et al. (2006) reported a long term rotation effect or suppression of Rhizoctonia bare patch with soft white wheat-barley and hard white-wheat-barley rotations indicating a beneficial effect of barley when grown in rotation with wheat.

Initial screening of cereal plants (oats and barley germplasm) for genetic resistance to *R. solani* AG-8 found some level of resistance or tolerance to the disease (McDonalds and Rovira, 1985), however, subsequent evaluation found only varying levels of susceptibility rather than resistance to the disease (Neate, 1989). Jitkov (1997) also evaluated 1,214 accessions from the USDA National Small Grains Barley collections for resistance to Rhizoctonia. Seventy-seven accessions were found to

display some level of resistance with one highly resistant accession CIho 8342. However, resistance of this line was not confirmed by further field testing (Wesselius, 2001).

Similarly, Smith et al. (2003) evaluated primary, secondary and tertiary gene pools of wheat to identify potential genetic sources of resistance to *Rhizoctonia* for use in cultivar improvement. Moderate levels of resistance were found in *Dasypyrum villosum*, a member of the tertiary gene pool, implying its usefulness as a potential source of gene for resistance to *R. solani* AG-8 in wheat. At present, there is no wheat and barley cultivar that is known to be genetically resistant to *R. solani* and *R. oryzae* (Smith et al., 2003) however, some level of tolerance to both pathogens has been reported in an EMS-induced wheat, Scarlet-Rz1 (Okubara et al., 2009). If no-till is to be successfully adopted in the Pacific Northwest, cereal genotypes that are resistant to *R. solani* AG-8 must be identified or developed. The continuous use of such resistant genotypes under any reduced tillage system can help reduce the populations of this fungal pathogen in the soil.

Sodium azide: An important chemical mutagen for mutation breeding.

Genetic variation upon which selection acts, has been defined as the ‘‘engine that propels plant breeding to meet its future challenges’’ (Zamir, 2001). Chemical mutagenesis is one of the tools that can be used to induce mutations in crop plants hence creating genetic variability needed for improving agronomic traits. Aside from creating genetic variation needed for cultivar improvement, the nature and functions of genes involved in crop growth and development can be studied using mutations in crop plants (Adamu, 2007). Different forms of favourable mutations in crops have been

induced using either chemical mutagens or ionizing radiations (Ahloowalia and Maluszynski 2001).

Sodium azide (NaN_3), an inorganic chemical mutagen which is commonly used in the laboratory as a respiratory inhibitor and vegetable and grain disinfectant has been reported to be a powerful mutagen. Its ability to cause mutations in higher plants was accidentally discovered when used as a respiratory inhibitor on barley plants (Spence, 1965) and has since been reported to be a potent mutagen in barley (Nilan et al., 1972; Sideris et al., 1973; Kleinhofs et al. 1974). Aside from barley, sodium azide has also been reported to be a potent mutagen in peas (Sander and Muehlbauer, 1977), soybean (Vig, 1973) and rice (Awan et al., 1980).

Studying the mutagenic effects of azide on barley plants, it was found to induce high mutation frequencies and 'negligible chromosomal abberations' demonstrating azide as an efficient mutagen (Konzak et al., 1972). The data obtained by Sander and colleagues (1978), also showed that sodium azide did not induce chromosomal aberations in embryonic shoots and microspores in barley confirming sodium azide as a ''point mutation mutagen.''

Sodium azide mutagenesis has produced traits of agronomic and economic importance in many crop plants. For example, in Europe, sodium azide was used to produce malting barley cultivars with resistance to powdery mildew, a damaging foliar disease of barley (Molina-Cano et al., 2003).

When compared to other potent mutagens, sodium azide is inexpensive, non-persistent and relatively safe to handle. The advantages of sodium azide thus provide the possibility of its use in providing numerous and useful mutant alleles for barley genetics, breeding and improvement (Nilan et al., 1972; Sander et al., 1978).

Cultivated barley (*Hordeum vulgare* subsp. *vulgare*) and available genepools

Barley, (*Hordeum vulgare* L.), is one of the earliest crops recorded to have been domesticated some 10,000 years ago in the Near East Fertile Crescent area of South-western Asia (Zohary, 1973; Zohary and Hopf, 2000). It is a member of the tribe Triticae, an important plant group to which other important temperate cereal crops such as wheat (*Triticum aestivum*), rye (*Secale cereale*) as well as other valuable forage grasses such as Russian wild rye (*Psathyrostachys fragilis*) and crested wheatgrasses (*Agropyron cristatum*) belong (von Bothmer, 1992). The tribe Triticae, is an evolutionary branch in the grass family, Poaceae and contains several species and genera. The genus *Hordeum* is widely distributed, mostly occurring in temperate areas of the world with Central and Southwestern Asia, Western North America, Southern South America and the Mediterranean serving as centers with the highest amount of native species (von Bothmer et al. 2003). In contrast to other genera within the tribe Triticae, *Hordeum* displays a high degree of biological diversity having both annual and perennial species. Similar to other species within the tribe Triticae, species within the genus *Hordeum* have a basic chromosome number of x=7 (von Bothmer, 1992). Diploids (2n=2x=14), tetraploids (2n=4x=28) and hexaploids (2n=6x=42) are the different ploidy types that occur within the genus (von Bothmer, 2003).

The loss of genetic diversity of the majority of the world's crops has resulted in an increased interest in the utilization of their wild relatives for crop improvement. As with most other crops, the genetic resources available for barley improvement have been classified into primary, secondary and tertiary gene pools (Harlan and de Wit, 1971) based on the ease of their utilization in breeding or crossability (von Bothmer et al., 1992).

Members of the primary gene pool include cultivated, wild and weedy forms of a crop species and the transfer of genes between members of this pool is easy. On the other hand, the transfer of genes of members of the secondary gene pool is possible but with some difficulty while the tertiary gene pool comprises species from which gene transfer is very difficult (von Bothmer et al., 1992).

The primary gene pool of cultivated barley includes a single species, *Hordeum vulgare* and comprises the breeding lines and released varieties, landraces and the progenitor of domesticated barley, *H. vulgare* subsp. *spontaneum* (von Bothmer et al., 1992). Members of this pool are cross-compatible, producing fertile and viable hybrids with no incompatibility or sterility barriers (von Bothmer et al., 1995; Singh, 2005).

The secondary gene pool also includes only a single species, *H. bulbosum* widely used in barley breeding for the production of doubled haploids through chromosome elimination (Pickering, 1984; Subrahmanyam and von Bothmer, 1987). Crosses of *H. bulbosum* to cultivated barley do occur but with difficulty as evidenced in reduced cross-ability and low seed fertility (von Bothmer et al., 1995). Progeny from crosses between *H. bulbosum* and *H. vulgare* produces plants with morphological traits similar to cultivated barley, however, they contain only haploid *H. vulgare* genome (Kasha and Kao, 1970). As a result, crosses between the two species have been exploited as a means of producing double haploids in barley for breeding purposes (Kasha and Kao, 1970; Arabi and Nabulisi, 2002). In addition to its use in double haploid production, *H. bulbosum* has also been identified as a potential source of useful traits such as abiotic stress tolerance, insect and disease resistance, self-incompatibility and perenniality that can be transferred to cultivated barley (Pickering and Johnston, 2005). However, despite its potential usefulness in barley improvement, several barriers such as chromosome instability, chromosome pairing and crossing over, and hybrid

infertility inhibit the transfer of useful genes to cultivated barley through hybridization (Pickering, 2000).

The remaining species of *Hordeum* constitute the tertiary gene pool and these cross with cultivated barley with great difficulty (von Bothmer et al., 2003) producing sterile hybrids thereby limiting their utilization for barley improvement (Henry, 2001). To date, there has not been the successful transfer of genes from members of the tertiary gene pool to cultivated barley (Pickering, 2000).

Wild barley: *Hordeum vulgare* subsp. *spontaneum*

Hordeum vulgare subsp. *spontaneum* C. Koch, the direct progenitor of present day cultivated barley (Harlan and Zohary, 1966), was first identified in Turkey by the German botanist, Carl Koch (von Bothmer et al., 2003). It has a brittle rachis, and it is a two-rowed diploid (2n=14) with an annual life cycle (Nilan, 1964). Although mostly self-pollinated, it exhibits a higher rate of cross-pollination than cultivated barley (Nevo, 1992).

Wild barley, *H. vulgare.* subsp. *spontaneum* occurs in its natural habitat in the Eastern Mediterranean also known as the Fertile Crescent beginning from Israel and Jordan in the southwest, extending to north and south of Turkey, Iraqi Kurdistan, and southwestern Iran (Harlan and Zohary 1966; Nevo 1992). It also occurs in central Asia such as Tibet, Turkmenia and Afghanistan (Nevo, 1992; Zohary and Hopf, 2000). Apart from its natural habitat, wild barley is also found on secondary, man-made habitats such as cultivated fields, road sides and abandoned fields (Nevo, 1992; Shen, 2010) where it mostly occurs as aggressive weeds (von Bothmer et al., 2003). It has also been found to occur in the summer-dry deciduous oak forests of the Middle East where it is an

important component of the open herbaceous vegetation characteristic of this region (Zohary and Hopf, 1993).

Wild *spontaneum* and cultivated barley show morphological similarities, however they can be distinguished by several characters. In contrast to cultivated barley with both two-rowed and six-rowed types, wild barley is exclusively two-rowed (von Bothmer et al., 2003). Spikes are characterized by a brittle rachis that disarticulates into single triplets with arrowheads at maturity. This character ensures easy shattering and dispersal of seeds in the wild (Nevo, 1992). Also, rachis segments possess long and tough bristles as well as tough awns that easily attach to animals as a means of ensuring seed dispersal (von Bothmer et al., 2003). A non-brittle rachis is a preferred trait that was selected for during the domestication of cultivated barley (von Bothmer et al., 2003). This ensures that at maturity, the spikes remain intact allowing for easy harvesting and threshing of seeds (Shen et al., 2010).

Another major morphological difference between the wild and cultivated barley is in the shape and size of the seeds. Seeds of wild barley are shrunken and not as plump as those of cultivated barley (von Bothmer and Komatsuda, 2011). In terms of adaptive traits, wild barley has a well-developed dormancy system (Takeda, 1995) that requires 'after-ripening' conditions necessary to prevent pre-harvest sprouting of mature grains (Gutterman et al., 1996).

H. v. subsp. *spontaneum* is cross-compatible and fully inter-fertile (Brown et al., 1978; von Bothmer and Hagberg, 1983) with cultivated barley. There are no crossing barriers between both forms, and natural hybridization is occasionally observed when both wild and cultivated forms are grown together (Harlan, 1976). Crosses (spontaneous or artificial) between both species produce fertile hybrids with normal

chromosome pairing and segregation during meiosis (Nevo, 1992; Zohary and Hopf, 2000).

The center of origin of a crop has been defined as that area where domestication occurred, and in which the wild progenitor and their cultivated forms coexist (De Candolle, 1959). Due to the evolution of wild relatives of crops across diverse ecological habitats, they have developed a wide range of diversity to many biotic and abiotic stresses (Nevo et al., 1986). The long-term co-existence with different pests within their natural habitat as well as the diverse biotic and abiotic components of the region has brought about the development of several useful genes necessary for the short and long-term survival of wild barley (Nevo, 1992; Nevo, 2007 (Harlan and deWet 1971)). These include genes for disease resistance, environmental stresses such as drought, heat and salt tolerance, and several morphological and agronomic traits of importance (Nevo, 1992).

Several studies have shown that wild barley harbors an abundance of useful alleles which can be utilized for improving traits of agronomic and economic importance that are absent in cultivated forms (Nevo, 1992; Ellis et al., 2000; Fetch et al., 2003).

There is a wide diversity of disease resistance in wild barley compared to cultivated barley (Nevo, 1992). Numerous sources of resistance to powdery mildew (Moseman et al., 1983; Fischbeck and Jahoor, 1991), Septoria speckled leaf blotch (SSLB) (Metcalfe et al., 1977; Toubia-Rahme et al., 1999; Fetch et al., 2003), leaf rust (Manisterski, 1986; Jin et al, 1995; Fetch et al., 2003) and leaf scald (Abbott et. al., 1992) have been identified. *H. spontaneum* has also been identified and utilized as a source of resistance against net and spot blotch (Sakti and Bailey, 1995; Sato and Takeda, 1997).

In a study carried out to assess the diversity of *spontaneum* accessions from Israel and Jordan for their reaction to six foliar fungal pathogens (Stem rust [caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn.], net blotch [caused by *Pyrenophora teres* Drechs f. *Teres*], spot blotch [caused by *Cochliobolus sativus* (Ito & Kurib.) Drechs. ex Dastur], Septoria speckled leaf blotch [caused by *Septoria passerinii* Sacc.] and powdery mildew [caused by *Blumeria graminis* f. sp. *hordei* Em. Marchal]) affecting cultivated barley in the United States and Canada, Fetch et al. (2003) observed high levels of diversity and heterozygosity for disease reaction in 116 accessions assessed at the seedling stage. Also, the diversity observed was high across and within collection sites of both locations. In addition, several major quantitative trait loci (QTL) have been identified in *spontaneum* conferring resistance to four of these diseases (net blotch, spot blotch, Septoria speckled leaf blotch and powdery mildew (Yun et al., 2005) and leaf scald [caused by *Rhynchosporium secalis* (Oudem.) J. J. Davis] (Genger et al., 2003).

Spring barley is an important annual crop in a 2 or 3-yr rotations with winter wheat, peas, lentils or fallow in the Pacific Northwest (PNW) of the United States (Smiley et al., 1992). Growers in this region are gaining increasing interest in no-till or direct seeding to reduce soil erosion, improve soil quality and reduce cost of production. No-till involves planting directly into the previous crop's residue or stubble without any form of tillage activity that stirs or turns the soil prior to planting (Cook et al., 2002). However, Rhizoctonia root rot and bare patch caused by *Rhizoctonia solani* AG-8 increases in no-till or direct seeding conditions (MacNish, 1985; Rovira, 1986; Pumphrey et al., 1987) and it is the most important disease of barley that is sown directly into cereal stubble in the PNW growing conditions (Pumphrey et al., 1987; Weller et al., 1986; Ogoshi et al., 1990). Also, barley is known to be more susceptible

to *R. solani* AG-8 than wheat and spring crops are generally more affected than crops sown in the fall (Smiley, 1996). Available control measures are less effective and host resistance appears to be the most promising method of controlling the disease. Many wheat, barley, oat, triticale, rye germplasm has been screened (McDonald and Rovira, 1985; Neate, 1989; Jitkov, 1997; Smith et al., 2003) to identify potential sources of resistance. However none of these exhibited true resistance to the disease and there are no known genotypes of wheat and barley that are resistant to the disease (Smith et al., 2003).

Based on the different studies indicating wild barley to be a rich source of resistance genes to important diseases of barley, it is possible that it could serve as a potential source of Rhizoctonia root rot resistance genes for use in cultivated barley improvement since there are no barriers to gene transfer between both forms. Wild barley accessions that show resistance or high levels of tolerance could serve as a potential gene donor for resistance to *Rhizoctonia solani* AG-8.

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CHAPTER TWO

MUTATION BREEDING AND GENETIC ANALYSIS OF RESISTANCE TO RHIZOCTONIA ROOT ROT IN BARLEY

Abstract

Direct seeding/no-till is a practice that is gaining importance among Pacific Northwest (PNW) growers in order to reduce soil erosion, improve soil quality, and decrease production costs. Spring cropping with no-till/direct seeding in the PNW offers a potentially better approach to increasing cropping intensity and circumventing the negative impacts tillage has on the environment, mainly on soil and water due to erosion. However, Rhizoctonia root rot and bare-patch caused by *Rhizoctonia solani* AG-8 is the most important disease affecting barley- an important spring crop in the PNW- in no-till/direct seeding systems. Options for managing the disease do not provide full protection, and commercial barley cultivars that are resistant to the fungus are not available. In an attempt to develop barley cultivars displaying true genetic resistance to the pathogen, we employed induced mutation as a means of creating new genetic variation. M_2 populations of sodium azide barley mutants of two different barley lines, 05WA-316.99 and 'Lenetah' were screened for disease reaction. Putative mutants were verified to confirm observed resistance. From these two lines, 10 putative M_3 individuals were identified and crossed to wild-type progenitor for genetic analysis. Evaluating the BC_1F_1 plants of verified putative mutants for disease reaction, a greater number of susceptible plants were observed based on disease severity ratings and other parameters measured. These findings suggest that resistance to Rhizoctonia root rot in mutant barley is inherited as a dominant trait.

INTRODUCTION

Barley (*Hordeum vulgare*) is the fourth largest cereal crop in terms of global production following maize (*Zea mays*), rice (*Oryza sativa*) and wheat (*Triticum aestivum*). In the Pacific Northwest (PNW) region of the United States, spring barley is an important rotational crop (Smiley et al., 1992) and is also well suited to the conservation tillage systems in this region (Schillinger et al., 2011). Barley is attacked by a number of pathogens that lower the productivity and cause economic losses. However in the PNW, the most important of these pathogens are the group of fungi that cause root, crown and stem diseases (Schillinger at al., 2011). With an increasing interest among PNW growers towards direct seeding systems in order to improve soil quality and save on production costs, the challenge of root diseases continues to be a major hindrance to the full adoption. Rhizoctonia root rot and bare-patch caused by *Rhizoctonia solani* AG-8 is the most important disease of barley under no-till or direct seeding systems in the PNW (Weller et al., 1986; Ogoshi et al., 1990). Although the disease is also known to affect wheat, barley is more affected and shows more stunting even under similar disease pressures (Paulitz and Steffenson, 2011).

The prevalence of the Rhizoctonia root rot and bare patch under direct seeding systems has been associated with volunteer crops and grassy weeds that increase the inoculum potential of the pathogen between harvest and subsequent planting (Smiley et al., 1992). Rhizoctonia root rot and bare patch is not a problem in conventional tillage systems, since tillage is thought to break up the hyhpal networks of the pathogen in the soil (Gill et al., 2002; Paulitz et al., 2002) or change the soil microbial population to those that are suppressive to the pathogen (Paulitz et al. 2002). However, in direct seeding systems, tillage is not an option for managing the disease. Given the wide host range of the pathogen, the use of crop rotation to manage the disease has met with little

success (Rovira, 1986; Cook, 2000). The use of biological control agents as an alternative to synthetic chemicals is an environmentally friendly option for the purpose of managing plant diseases. These organisms control plant diseases by suppressing the activities and/or populations of plant pathogens (Pal and Gardener, 2006). The biological control activity of *Bacillus* spp. against the three groups of pathogens causing take-all (caused by *Gaeumannomyces graminis* var. *tritici*), Pythium root rot (caused by *Pythium irregulare* and *P. ultimum*) and Rhizoctonia root rot when applied as seed treatments in controlled environment and under direct seeding systems was investigated (Kim et al., 1997). Strains of this species were found to be effective against Rhizoctonia root rot in growth chamber assays, causing significant yield increases under field trials involving direct seeded cereals. In spite of the success recorded with this organism in suppressing Rhizoctonia root rot, these strains are not available for commercial usage (Paulitz et al., 2002). Using the transgenic approach, Wu et al. (2006), showed that purified endochitinase *ThEn42* from the filamentous fungus *Trichoderma harzianum* strongly inhibited the growth of both *R. solani* AG-8 and *R. oryzae* thereby providing another means for managing the disease. However, concerns over public acceptance of transgenic crops have generally limited their widespread deployment on a commercial scale, especially among small grain cereals.

Based on the observation that limited options are available for managing the disease, host resistance appears to be the most promising approach. A total of 1,214 accessions from the USDA National Small Grains Barley collections were assessed for resistance to the pathogen, with one accession, CIho 8342 displaying the highest level of resistance (Jitkov, 1997). However, further field testing did not confirm the resistance of this line (Wesselius, 2001). In the case of wheat, another important cereal

crop in the PNW that is also attacked by the pathogen, no true genetic resistance has been identified in different germplasm that have been screened (Smith et al., 2003).

Identifying barley genotypes with resistance to Rhizoctonia root rot would be of great benefit to PNW growers for several reasons. Firstly, even though barley is more susceptible than wheat under similar disease pressures, it has been found that barley grown in rotation with wheat under direct seeding systems causes a long term reduction in bare patch disease (Schillinger and Paulitz, 2006). In addition to the role of barley in suppressing diseases, it is also known to improve the productivity of wheat when included as a rotation crop with winter wheat (Cook and Veseth, 1991). Therefore, it is evident that with the use of *Rhizoctonia* resistant barley genotypes in rotation with winter wheat under any direct seeding system, a significant rotation effect can be achieved within a shorter period of time through a reduction in the inoculum of *R. solani* AG-8 from soil. This will provide growers with a sustainable, environmentally sound, and a more generally acceptable measure for managing the disease in the PNW. Based on the observation that available control measures for combating Rhizoctonia root rot in the PNW are less efficient, and the absence of true host resistance in the crop germplasm available to breeders, it is important that an efficient, yet non-transgenic alternative to managing an intractable disease like Rhizoctonia root rot and bare patch be developed. Inducing host resistance in barley through the application of mutations is one such alternative.

Genetic variation is the primary tool needed for any successful breeding or crop improvement program, and mutations are known to be the ultimate source of genetic variation. Induced mutations are of great interest to plant breeders because they provide new sources of genetic variation from which plant breeders can select genotypes with useful and economically important traits such as increased yield, improved plant

architecture, and disease resistance. More importantly, when an important trait such as resistance to diseases of economic importance like Rhizoctonia root rot has not been identified in the germplasm available to breeders, the use of induced mutations might provide plants with resistance to the disease. Conventional methods of inducing mutations in crops involve the use of either physical mutagens like radiation or chemical mutagens. Various kinds of useful mutations have been induced in crops using both methods (Ahloowalia and Maluszynski, 2001). Sodium azide (NaN_3) is an inorganic compound that has been reported to be a potent mutagen in barley (Nilan et al., 1972; Kleinhofs et al., 1974). It induces high mutation frequencies with virtually no chromosomal damage or aberration (Konzak et al., 1972). Notable among its usefulness in creating useful mutations is its induction of powdery mildew resistance in two and six-rowed malting barley cultivars (Molina-Cano et al., 2003).

In an attempt to develop barley genotypes that show true genetic resistance to Rhizoctonia root rot, chemical mutagenesis using sodium azide was used in this study to create genetic variation in selected PNW-adapted barley varieties with the objective of identifying putative mutants that exhibit resistance to the disease. Since the overall goal of the research study is to develop barley cultivars that can be released to PNW farmers to manage the disease, it is important that the genetics of resistance to Rhizoctonia root rot in barley be understood. Towards this goal, the study went on further into confirming the resistance in the progeny of putative mutants, carrying out a series of backcrosses to the wild-type progenitor, and evaluating the progeny obtained from the crosses in order to gain a better understanding of how the resistance trait is inherited.

MATERIALS AND METHODS

Mutagenic treatment of plant material

Seeds of the two-row spring barley cultivar Lenetah (Obert et al., 2008) and breeding line 05WA-316.99 were treated with the chemical mutagen, sodium azide (NaN_3). Mutagenic treatment was carried out using the modified procedure described by Kueh and Bright (1981). About 0.5 kg seeds of both Lenetah and 05WA-316.99 were pre-soaked for 16 h at 0 °C and transferred to a water bath for a second pre-soaking at 20 °C for 8 h with aeration. Pre-soaked seeds were then treated with 1mM of sodium azide in 0.1 M phosphate buffer (pH 3.0) for 2 h. The treated seeds were rinsed three times in tap water followed by a 30 min wash in running tap water, and dried under a hood overnight. Sodium azide treated seeds referred to as M_1, were sown at the Washington State University Spillman Agronomy Farm, Pullman WA to obtain selfed progeny (M_2). These M_2 seeds were obtained by bulk-harvesting the spikes of the M_1 plants. M_2 seeds of 'Lenetah' and '05WA-316.99' were screened for resistance/tolerance to Rhizoctonia.

Inoculum production

Rhizoctonia solani AG-8 isolate C1 (Weller et al., 1986) was used to assess disease reactions. Inoculum preparation and enumeration were carried out as described by Paulitz and Schroder (2005) in which 250 ml of oat grain soaked in 250 ml of distilled water in 1-liter flask was autoclaved for 90 min at 120 °C. This was done twice at a 24 hr interval (Ogoshi et al., 1990). The second autoclaving was necessary in order to eliminate microbes with heat resistant spores that may have survived the first autoclaving. *R. solani* AG-8 isolate C1 maintained on potato dextrose agar was used to inoculate the autoclaved oat seeds. This was done by transferring ten 1-cm agar plugs

from 1-wk-old potato dextrose agar culture of *R. solani* AG-8 into flasks containing the autoclaved oat seeds. Inoculated oats were incubated for 4 wk at room temperature and shaken once every week to allow for an even distribution of colonized oat seeds. At the end of 4 wk, colonized oat seeds were dried under a laminar flow for 2 d, after which they were thoroughly mixed and a small portion ground with a coffee grinder and sieved to obtain particles of sizes between 250 and 1000 µm. To determine the concentration of inoculum, 100 mg of sieved inocula was suspended in 5 ml of sterile water from which a 10-fold and 100-fold dilution of the suspension was made. From each of the suspensions, 200 µL were plated onto *Rhizoctonia* selective media (water agar containing 100 µg/mL chloramphenicol and 1 µg/mL a.i benomyl) and incubated at room temperature for 48-72 hr, after which *Rhizoctonia* colonies were counted. Inoculated oat seeds were stored at 4 °C and ground when needed.

Screening Procedure

Thatuna silt loam soil obtained from the Washington State University Spillman Agronomy Farm was sieved and pasteurized at 60 °C moist heat before use. A concentration of 100 propagules per gram (ppg) of inoculum was mixed with a portion of the soil while the other portion (pasteurized only) served as control for comparing healthy and diseased plants. Mixing of inoculum and soil was manually done in plastic bags. Infested soils were poured into flats and watered to near saturation. Non-infested soils were also put into flats and watered to near saturation.

M_2 seeds of both Lenetah and 05WA-316.99 were sown into the flats containing infested soil and covered with polythene plastic until emergence. Wild-types (non-mutagenized seeds) of both progenitor lines were planted into 2 separate flats containing infested and non-infested soils to serve as positive and negative controls

respectively. Each flat contained 200 seeds. A total of 10,500 and 10,200 seeds were sown for breeding line 05WA316.99 and cultivar Lenetah respectively. Flats were placed in a growth room with 15 °C day and night temperatures, 14 hr photoperiod and 80% RH. At 21 d after planting (DAP), putative mutants showing some levels of tolerance/resistance were rescued and transplanted in a commercial potting mix in the greenhouse to obtain M_3 seeds for rescreening. Selection of putative mutants was done by visual observation for plants with no yellow colorations on leaf tips and plants with greater shoot length when compared with the positive control, and similar in height to the negative control plants. In addition, roots of selected plants were washed free of soil and examined for disease symptoms as compared with roots of plants grown in non-infested soil. Only plants with no disease symptom on roots were selected and transplanted to the greenhouse.

Verification of putative M_2 individuals

To confirm the resistance of selected plants and reduce the chances of selecting and advancing plants that might have escaped disease, all M_3 seeds from selected M_2 plants were subjected to similar screening procedures as the M_2 seeds. A total of 73 M_3 seeds from the 2 putative M_2 plants were retested. Autoclaved soil from the same source was infested with 100 ppg of inoculum and thoroughly mixed in polythene bags. In this case, each M_3 seed was sown in plastic conetainers (Stuewe & Sons, Corvallis, OR, USA), watered to near saturation and placed in a Conviron™ (Pembina, ND, USA) growth chamber at conditions of 80% humidity, 15 °C day and night temperatures and 14 h/day lighting.

At 21 DAP, roots of each M_3 plant were washed and each plant was evaluated for plant height, fresh seedling biomass, and disease severity on seminal and crown

roots. Evaluation of disease severity on roots was done on a scale of 0-8 with the following criteria: 0 = no lesions, 1 = <50% of the roots with a single lesion, 2 = <50% of the roots each with a few lesions, 3 = >50% of the roots each with one or more lesions, 4 = <50% of the roots with lesions within 1 cm of the seed, 5 = >50% of the roots with lesions within 1 cm of the seed, 6 = >50% of the roots with terminal lesions less than 3 cm from the seed, 7 = >50% of the roots with terminal lesions less than 1 cm from the seed, and 8 = 100% of the roots with terminal lesions <1 cm from the seed (Kim et al., 1997). Selection for resistant plants using this scale was based on the grouping: highly resistant (0-0.9), resistant (1.0-3.0), moderately resistant (3.1-4.0), moderately susceptible (4.1-5.0), susceptible (5.1-6.0) and highly susceptible (6.1-8.0) (Smith et al., 2003)

M_3 plants showing greater shoot lengths, higher seedling and root weights, and disease ratings of between 0 and 3 were rescued and transferred to the greenhouse for crossing. Reciprocal crosses of each putative M_3 with the wild-type progenitors were made to obtain BC_1F_1 seeds.

Inheritance studies

Seeds from each cross (BC_1F_1) of putative M_3's were screened for resistance to Rhizoctonia root rot as described above using similar growth chamber conditions. At the end of 3 weeks, all BC_1F_1 plants were harvested, roots of each plant were washed and assessed for disease symptoms, and the fresh seedling biomass of each individual plant was taken after which they were transplanted in commercial potting mix in the greenhouse where they were advanced to BC_1F_2.

RESULTS AND DISCUSSION

M_2 population screening and verification experiment

Lenetah: Out of 10,200 M_2 seeds that were assayed, five seedlings were identified as resistant and rescued for retesting. From the verification experiment/retesting, two different M_2 individuals (designated as MLenetah_1 and MLenetah_2 respectively) were resistant to Rhizoctonia root rot based on the results obtained when their M_3 progeny were assayed for resistance to the fungus. From a total of 45 M_3 progeny of these putative M_2 individuals, six plants (MLenetah_1.1, MLenetah_1.2, MLenetah_1.3, MLenetah_1.4, MLenetah_1.5 and MLenetah_2.1 from the first and second M_2 plants respectively) displayed resistance. All six plants had disease severity ratings of between 0 and 3. However, only four of these M_3 individuals (MLenetah_1.1, MLenetah_1.2, MLenetah_1.3 and MLenetah_2.1) were used for reciprocal crosses with wild-type Lenetah. The two M_3 individuals that were not used for crosses had a dwarf architecture with abnormal spikes.

05WA-316.99: From a total of 10,500 M_2 seeds that were screened for resistance to *R. solani* AG-8, 10 M_2 plants displayed resistance based on visual observation for greater plant height, and no disease symptoms on the roots. These were rescued and transplanted in commercial potting mix in the greenhouse to obtain M_3 seeds for retesting. From the verification experiment/retesting, two different M_2 individuals (designated as M05WA_1 and M05WA_2 respectively) showed resistance to Rhizoctonia root rot when screening 73 M_3 progeny for resistance to the fungus. From these, six M_3 plants (M05WA_1.1, M05WA_1.2 and M05WA_1.3 from the first putative M_2 plant and M05WA_2.1, M05WA_2.2 and M05WA_2.3 from the second putative M_2 plant) showed resistance. Plants M05WA_1.1, M05WA_1.2, M05WA_2.1, and M05WA_2.2 had disease ratings of between 0 and 3 while M05WA_1.3 and

M05WA_2.3 had disease ratings of 4 and were selected because they displayed greater plant height and fresh seedling biomass comparable to plants grown in non-infested soil. Reciprocal crosses involving these six M_3 individuals and the wild-type parent (05WA-316.99) was carried out in the greenhouse to obtain BC_1F_1 seed. Seeds obtained from all crosses were screened for resistance to the fungus.

BC_1F_1 screening experiment: For both 'Lenetah' and 05WA-316.99, partial sterility of all M_3 plants used in the crosses was observed. Blank florets were also observed from selfed seeds obtained from these putative M_3 plants. For 'Lenetah', all the crosses that involved the four putative M_3 plants did not set seeds. The partial sterility observed could be an explanation for the inability of the crosses to produce seeds. Greenhouse condition is another possible factor that could have resulted in the low seed set. Therefore, no BC_1F_1 seed was screened for this cultivar. On the other hand, for 05WA-316.99, a total of 98 seeds from all the crosses were evaluated for resistance to the fungus. On the basis of disease severity ratings, a wide range of disease reaction was observed among the BC_1F_1 plants evaluated for this line. Seven of the plants were rated resistant (ratings of 1-3).

To date, true genetic resistance to Rhizoctonia root rot has not been found in barley. Using induced mutations, we were able to identify plants that displayed resistance to the disease. Also, we carried out a second screening to validate the resistance observed in the putative mutants. Although we did not carry out genetic analysis beyond the first generation of backcrosses, the observation that all the BC_1F_1 plants evaluated were not susceptible rules out the possibility that resistance is inherited as a recessive trait. Also, the greater percentage of susceptible plants in the BC_1F_1 screening experiment excludes the possibility of complete dominance. However, with the relatively low amount of seeds obtained and evaluated from each cross, the above

conclusions are at best speculations. Jitkov (1997) studied the inheritance of resistance to *R. solani* AG-8 by analysing the F_2 progeny obtained from crosses between selected resistant and susceptible accessions identified from the USDA National Small Grains Barley core collection. Based on his results, resistance to the fungus was either controlled by single or many genes. However, subsequent testing contradicted the resistance initially identified. Okubara and colleagues (2009) reported a single gene model for tolerance to *Rhizoctonia* in an EMS-induced wheat mutant. Therefore, evaluating the segregating BC_1F_2 population for their reaction to the fungus and carrying out chi-square analysis to test different models of inheritance would provide information on pattern of inheritance of the resistance observed in this study.

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CHAPTER THREE

IDENTIFICATION OF RESISTANCE TO RHIZOCTONIA ROOT ROT IN WILD BARLEY (*Hordeum vulgare* subsp. *spontaneum*)

Abstract

Rhizoctonia root rot and bare-patch caused by *Rhizoctonia solani* AG-8 limits crop yields in no-till/direct seeding systems. In the Pacific Northwest (PNW) region of the United States, the disease serves as an important hindrance to the widespread adoption of this cropping system. Available control measures do not eliminate the pathogen from the soil and also do not significantly increase yield. Host resistance offers a better approach for managing the disease. However, currently there is no resistant germplasm available. In the PNW, spring barley is an important rotational crop with winter wheat, peas and lentils. The role of barley as a rotational crop highlights the possibility for its use in managing the disease if resistant barley cultivars are included in the normal 2 to 3 year rotations with winter wheat under reduced tillage. Therefore, the objective of our research was to identify potential sources of resistance to *R. solani* AG-8 in barley. Since there is no known resistance in the natural populations of barley available to breeders, we evaluated 317 *Hordeum spontaneum* accessions that make up the Wild Barley Diversity Collection. Accessions were assessed for resistance based on disease severity ratings, shoot length, fresh root weigh and fresh seedling weight in a controlled environment. Most of the accessions evaluated were in susceptible rating categories. However, a single accession, WBDC 021 was identified that displayed moderate resistance to the pathogen, and four additional accessions were considered moderately resistant to moderately susceptible. Field testing is still required to be absolutely certain of any useful level of resistance.

INTRODUCTION

Rhizoctonia root rot including bare-patch caused by the fungal pathogen *Rhizoctonia solani* Kühn AG-8 (teleomorph: *Thanatephorus cucumeris* Frank Donk) (Neate and Warcup, 1985), are important diseases that limit crop yields in no-till/direct seeding systems in the Pacific Northwest (PNW) region of the United States (Weller, 1986). The disease was first reported in Australia in the late 1920s (Samuel, 1928) and later identified as a serious problem in direct seeded fields of wheat and barley in the PNW in 1984 (Weller, 1986). In the PNW, traditional seed bed preparation and weed control measures involve intensive tillage operations which bring about a significant loss in topsoil due to wind and water erosion in the low and high precipitation zones respectively. As a result, growers in the PNW have been shifting towards reduced tillage and no-till/direct seeding systems to improve overall soil quality, decrease environmental pollution and lessen production costs. Reduced tillage systems ensure at least 30% residue cover on the soil surface before planting. In no-till/direct seeding, which is the extreme form of any reduced tillage system, seeds are sown directly into the residue of the previous crop without any form of tillage activity that stirs or mixes the soil prior to planting (Cook et al., 2002). However, Rhizoctonia root rot and bare patch increases with reduced tillage (Rovira, 1986; Pumphrey et al., 1987) and is also known to pose serious threats to crop yields in the first few years of conversion from conventional tillage systems to no-till (Schroeder and Paulitz, 2006), thereby, serving as a major hindrance to the widespread adoption of no-till in this region (Schroeder and Paulitz, 2006). Spring cropping with no-till/direct seeding offers a potentially better approach to increasing cropping intensity and circumventing the negative impacts of tillage on the environment, mainly on soil and water due to erosion (Papendick, 1998). Furthermore, it has been shown that barley in rotation with winter wheat improves the

productivity of winter wheat (Cook and Veseth, 1991).For spring barley (*Hordeum vulgare*), an important annual crop in a 2 or 3-yr rotations with winter wheat (*Triticum aestivim*), peas (*Pisum sativum*), lentils (*Lens culinaris*) or fallow in the PNW (Smiley et al., 1992), Rhizoctonia root rot appears to be the most important disease under direct seeding systems (Weller et al., 1986; Pumphrey et al., 1987; Ogoshi et al., 1990). Spring barley is also known to be more susceptible than wheat (*Triticum aestivum*) under similar inoculum levels (Paulitz and Steffenson, 2011). Affected plants show significant stunting with characteristic brownish lesions on the roots and rotted root tips having sharp, pointed tips usually referred to as 'spear tips' (MacNish and Fang, 1987). The most severe form of the disease, referred to as 'bare patch' is observed on direct seeded fields in which affected plants appear severely stunted in patches on the field.

Available agronomic and cultural control measures do not eliminate the pathogen from the soil. Therefore, host resistance appears to be the most promising method for managing the disease. The continuous use of resistant cultivars under any reduced tillage system can help reduce the populations of this fungal pathogen in the soil. However, many wheat, barley, oat (*Avena sativa*), triticale (*Triticale hexaploide*) and rye (*Secale cereale*) germplasm have been screened (McDonalds and Rovira, 1985; Neate, 1989; Jitkov, 1997; Smith et al., 2003) to identify potential sources of resistance but none of these exhibited true resistance. At present, there are no genotypes of wheat and barley that show resistance to Rhizoctonia root rot (Smith et al., 2003) although, some level of tolerance to has been reported in an ethyl methane sulphonate-induced mutagenized wheat, Scarlet-Rz1 (Okubara et al., 2009).

Hordeum vulgare subsp. *spontaneum* C. Koch (2n=2x=14), the direct progenitor of cultivated barley (von Bothmer et al., 2003) is a rich source of useful alleles that can

be deployed to improve agronomic and economic traits of interest absent in cultivated barley (Nevo, 1992). Being a member of the barley primary gene pool (von Bothmer et al., 1992), it crosses easily with cultivated barley (Brown et al., 1978; von Bothmer and Hagberg, 1983) producing fertile hybrids (Nevo, 1992; Zohary and Hopf, 2000). Several studies have identified *H. vulgare* subsp. *spontaneum* accessions native to the Fertile Crescent as well as other areas that show high levels of resistance to important diseases of barley such as powdery mildew [caused by *Blumeria graminis* f. sp. *hordei* Em. Marchal], leaf scald [caused by *Rhynchosporium secalis* (Oudem.) J. J. Davis], septoria speckled leaf blotch [caused by *Septoria passerinii* Sacc.], net blotch [caused by *Pyrenophora teres* Drechs f. *Teres*], spot blotch [caused by *Cochliobolus sativus* (Ito & Kurib.) Drechs. ex Dastur], stem rust [caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn.], leaf rust (*Puccinia triticina* Eriks) and stripe rust (*Puccinia. striiformis* f. sp. *tritici*) (Moseman et al., 1983; Abbott et al., 1992; Sakti and Bailey, 1995; Sato and Takeda, 1997; Fetch et al., 2003; Steffenson et al., 2007). At the same time, several major quantitative trait loci (QTL) have been identified in *H. vulgare* subsp. *spontaneum* conferring resistance to net blotch, spot blotch, Septoria speckled leaf blotch, powdery mildew (Yun et al., 2005) and leaf scald (Genger et al., 2003).

To date, wild species of barley have not been explored for potential resistance to Rhizoctonia root rot. Based on the findings that *Hordeum vulgare* subsp. *spontaneum* harbours useful alleles for resistance to many diseases of barley, it is possible that wild barley could harbor useful genes conferring resistance to Rhizoctonia root rot. Since there is no hindrance to the transfer of disease resistance genes present in wild barley to their cultivated forms, resistant wild barley accessions can serve as a gene donor source for managing the disease. The important role of barley as a rotational crop in the PNW highlights the possibility for its use in managing the disease if resistant barley cultivars

are included in the normal 2 to 3 year rotations with winter wheat under reduced tillage. The objective of this study was to identify potential sources of resistance to Rhizoctonia root rot in a collection of wild barley accessions collected from the Fertile Crescent and other areas.

MATERIALS AND METHODS

Wild Barley Diversity Collection (WBDC)

Three hundred and seventeen accessions of wild barley (*H. vulgare* L. subsp. *spontaneum*) provided by Brian J. Steffenson, Department of Plant Pathology, University of Minnesota were initially screened for levels of susceptibility to *Rhizoctonia solani* AG-8 isolate C1. These accessions represent the Wild Barley Diversity Collection assembled by Dr Jan Valkoun (Barley Curator (retired), International Center for Agricultural Research for Dry Areas (ICARDA), Aleppo, Syria) and Dr. Brian Steffenson (University of Minnesota, USA). Assemblage of the these accessions into the Wild Barley Diversity collection was done on the basis of several eco-geographical factors including longitude/latitude, rainfall, temperature, elevation and soil type (Steffenson et al., 2007). The accessions are from 20 different countries with the majority of them from the Fertile Crescent (77.4%) and the others from North Africa (3.8%), central Asia (15.7%), and the Caucasus region (2.8%) (Table 1) (Steffenson et al., 2007). Accessions showing resistance in the first screening were verified in the second screening.

Preparation of Rhizoctonia inoculum and soil infestation

Rhizoctonia solani AG-8 isolate C1 (Weller et al., 1986) was used for both the initial and second screening/ verification experiment. Rhizoctonia inoculum preparation and enumeration were prepared as described by Paulitz and Schroeder (2005) in which

250 ml of oat grain soaked in 250 ml of distilled water in 1-liter flask was autoclaved twice for 90 min at 120°C. The second autoclaving was done 24 hr (Ogoshi et al., 1990) after the first autoclaving to eliminate microbes with heat resistant spores that may have survived the first autoclaving. Inoculation was done by transferring 10 1-cm agar plugs from an approximately 1-week- old potato dextrose agar culture of *R.solani* AG-8 into flasks containing the autoclaved oat seeds. Inoculated oat seeds were incubated at room temperature for 4 weeks and shaken once every week to allow for an even distribution of colonized oat seeds. At the end of 4 weeks, colonized oat seeds were dried under a laminar flow for 2 d, after which they were thoroughly mixed and a ground with a coffee grinder and sieved to obtain particles of sizes between 250 and 1000 µm.

For enumeration of inocula, 100 mg of sieved inocula was suspended in 5 ml of sterile water from which a 10-fold and 100-fold dilution of the suspension was made. 200 µL of each of the suspensions were plated onto *Rhizoctonia* selective media (water agar containing 100 µg/mL chloramphenicol and 1 µg/mL a.i benomyl) and incubated at room temperature for 48-72 hr. after which *Rhizoctonia* colonies were counted. Inoculated oat seeds were stored at 4°C and ground when needed. A single concentration of 100 propagules per gram (ppg) was used for the entire experiment. Thatuna silt loam soil collected from Spillman Agronomy Farm, Pullman WA was sieved to ensure particle size uniformity and pasteurized at 60°C for 30 min (to eliminate antagonistic micro-organisms) before use.

Screening for resistance to Rhizoctonia root rot

For both the initial screening and second screening experiments, 3.8 cm x 20.5 cm (2.5 cm x 16.6 cm) plastic conetainers (Stuewe & Sons, Corvallis, OR, USA), were plugged at the bottom with cotton balls and filled with about 50 g of inoculated soil

before planting. A single seed of each *H. spontaneum* accession was placed in each soil-containing conetainer after which the seeds were covered with about 10 g of non-inoculated soil and watered. Since there are no known resistant cultivars, seeds of each accession were also planted in non-inoculated soil to serve as untreated checks and a basis for comparison. Also, a barley cultivar, Lenetah was included as a susceptible check. For the verification experiment, barley cultivars, Bob, Lenetah and and breeding line 05WA-316.99 were included as susceptible checks. The conetainers were arranged in a completely randomized design with each accession replicated 10 times, covered with plastic polythene bags to ensure rapid disease development and to reduce evaporation losses from the soil, and placed in a Conviron (Pembina, ND, USA) growth chamber at conditions of 80% humidity, 15°C day and night temperatures, and 14 h/day lighting for 21 d. Plants were watered every 2 d before and after emergence. At 21 days after planting (DAP), individual plants were gently removed from the conetainer and the roots of each plant were washed free of soil. Each plant was evaluated for shoot length, fresh seedling weight, fresh root weight and disease severity on seminal and crown roots. Evaluation of disease severity on roots was carried out as described by Kim and colleagues (1997) using a disease rating scale of 0-8 where 0 = no lesions, 1 = <50% of the roots with a single lesion, 2 = <50% of the roots each with a few lesions, 3 = >50% of the roots each with one or more lesions, 4 = <50% of the roots with lesions within 1 cm of the seed, 5 = >50% of the roots with lesions within 1 cm of the seed, 6 = >50% of the roots with terminal lesions less than 3 cm from the seed, 7 = >50% of the roots with terminal lesions less than 1 cm from the seed, and 8 = 100% of the roots with terminal lesions <1 cm from the seed.

Data Analysis

Both the initial and second growth chamber screening experiments were analyzed as a two-way analysis of variance (ANOVA) arranged in a completely randomized design. The treatments include Accessions (n=317 and 14 in first and second experiment, respectively) and *Rhizoctonia solani* (presence and absence of Rhizoctonia inoculum). Each accession was replicated 10 times per treatment combination. Before conducting ANOVA, tests for homogeneity of variance and normality were carried out. The Box-cox procedure was used in determining the appropriate lambda for transformation of the data because data were not distributed normally. ANOVA was carried out on transformed data to test for significance of accession and Rhizoctonia inoculum main effects, as well as, significant interactions between main effects. Data were analysed using the GLM procedure in SAS version 9.2 (SAS Institute Inc, Cary, NC). Six of the accessions that were rated either resistant or moderately resistant and the five that were rated moderately susceptible in the first screening were re-tested and analysed as described for the first screening. Due to significant interaction between main effects, inoculum treatment and non-inoculated controls were compared for each accession using *t* tests and the difference between both was considered significant at $P < 0.05$. Back-transformed estimates of least square means (LSM) obtained from ANOVA were used for tables and graphs with the exception of the graph showing distribution of accessions based on disease severity ratings. Correlation analysis using Pearson's correlation coefficient was calculated to determine the correlation between disease severity ratings, shoot length, fresh root weight and fresh seedling weight.

RESULTS AND DISCUSSION

First screening

For all the growth parameters measured, both accession and *Rhizoctonia* main effects were significant at $P < 0.0001$ (Table 2). A significant interaction between accessions and *Rhizoctonia* inoculum was also observed at the same level of significance for all the growth parameters. Also, significant differences ($P < 0.05$) for the growth parameters among accessions in the absence of *Rhizoctonia* inoculum were observed. The probable reason for the observed significant interaction could be the differences in origin as well as genetic differences among the accessions which are manifested in the different reaction response to Rhizoctonia root rot. Disease severity ratings of the entire 317 accessions ranged from 1.6 - 8.0 with a mean disease rating of 5.6 indicating that most of the accessions were susceptible. In the absence of *Rhizoctonia* inoculum, all accessions had ratings of 0. Using the disease ratings, accessions were divided into 5 groups of resistant (1.0-3.0), moderately resistant (3.1-4.0), moderately susceptible (4.1-5.0), susceptible (5.1-6.0) and highly susceptible (6.1-8.0) (Smith et al., 2003) accessions (Fig 1). Based on this grouping, less than 2% of the entire accessions showed resistance with WBDC 021 rated as resistant and accessions WBDC 005, 007, 013, 300 and 345 rated as moderately resistant. About 30 percent of the accessions were moderately susceptible and the remainder were either susceptible or highly susceptible. The susceptible check Lenetah that was included in the experiment as a susceptible check fell in the highly susceptible group with a mean disease rating of 7.4.

With the exception of disease ratings, significant differences among accessions in the absence of *Rhizoctonia* inoculum were observed for shoot length, root weight and seedling weight. Because of the differences among accessions in the absence of the

Rhizoctonia inoculum, it was not possible to make comparisons between accessions with respect to the growth parameters measured. Therefore an analysis to test (at $P < 0.05$) within each accession, the effect of the *Rhizoctonia* inoculum was carried out. Only results for accessions in the resistant, moderately resistant group and a few accessions in the moderately susceptible group are reported. Of the 317 accessions that were screened for resistance to *R. solani*, 24, 63 and 25 accessions had shoot length, root weight and seedling weight, respectively, that were not significantly different from the non-inoculated control.

R. solani AG-8 did not cause a significant reduction in the shoot length and root weight of WBDC 021 that was rated resistant (Table 3). However, a significant reduction in fresh seedling weight was observed for this accession in the presence of *R. solani* AG-8. Three of the accessions in the moderately resistant group (WBDC 005, 007 and 013), plants grown on infested soils did not differ significantly from the non-inoculated controls for one or more of the parameters measured. For the remaining 2 accessions rated moderately resistant (WBDC 300 and 345), *R. solani* caused a significant reduction in shoot length, fresh seedling and root weight. All the accessions rated resistant and moderately resistant were re-tested to verify the resistance observed in the first screening. For accessions in the moderately susceptible group, *R. solani* caused a significant reduction in the one or more of the parameters measured. However, for many of the accessions in this group, the amount of shoot length, root weight or seedling weight produced when infested with the pathogen was not significantly different from the control. It was imperative that we came up with a measure for selecting accessions within this group that could be re-tested. Therefore, accessions with moderately susceptible disease ratings, but close to the moderately resistant cut off value (that is accessions having disease severity ratings of between 4.1 and 4.5) and

which did not differ significantly from the control for one or more of the parameters measured were selected. Based on this, accessions WBDC 009, 079, 080, 085 and 103 were re-tested together with the other accessions in the resistant groups.

In the first screening, for the majority of the accessions rated susceptible or highly susceptible, it was observed that the difference between inoculum treatment and control was not significant for one or more of the growth parameters measured. These plants might have been given high disease scores based on the visual disease symptoms observed on the roots but they did not suffer from either reduced shoot or root development. Similarly, some of the accessions that might have been rated as resistant using the disease rating scale showed significant reduction in one or all of the growth parameters. A few of the accessions were also observed with higher seedling weights in infested soil compared non-infested soils (Table 3). These observations suggest that although disease ratings might offer a quick and easy method of assessing resistance when screening high numbers of genotypes, the method is quite subjective and may not offer the best approach for selecting desirable genotypes. Other methods such as evaluating the total root biomass produced, especially when disease symptoms are severe as was observed in this study and the total root length (Higginbotham et al., 2004; Okubara and Jones, 2011) in the presence of the pathogen might provide useful information on which genotypes are resistant or tolerant.

Relatively high and significant correlations for all the parameters measured were observed in both initial and second screening experiment (Table 4). In the first experiment, there was a high correlation between the disease severity ratings and shoot length, root weight, and seedling weight while a much higher degree of correlation was observed in the second screening. Disease severity rating was negatively correlated with shoot length, root weight and seedlings weight, and a positive correlation was observed

between all other growth parameters. The negative correlations (-0.67 and -0.90 in first and second experiment respectively) between disease severity rating and shoot length are consistent with the work of Schroeder and Paulitz (2008) and Babiker et al (2011). Schroeder and Paulitz found a significant negative correlation (-0.66) between plant height and disease rating while Babiker and colleagues observed a much higher magnitude of correlation (-0.94, -0.92 and -0.99) in the three years in which the experiment was conducted respectively for shoot length and disease rating.

Second screening

From the ANOVA of the second screening results, there was a significant *Rhizoctonia* inoculum and accession effect at P <0.0001 (Table 5). At the same level of significance, the interaction between *Rhizoctonia* inoculum and accession was also significant. Disease severity ratings ranged from 3.6 to 7.1 with a mean rating of 4.9. Accession WBDC 021 that was rated resistant with a mean disease score of 1.6 in the initial screening was rated moderately resistant with a mean disease rating of 3.6 in the second screening. All the 5 accessions that were rated moderately resistant in the first experiment were re-rated as either moderately susceptible or susceptible based on their disease severity ratings. Accession WBDC 103 having a disease score of 4.5 in the first screening was given a score of 3.9 in the second screening, which puts it in the moderately resistant category. Summarizing the two screening experiments, several lines showed consistency or reduction in disease ratings within or near the 3.9 upper limit for the moderately resistant rating (WBDC 005 [3.7 – 4.0], WBDC 009 [4.2 – 4.3], WBDC 085 [4.4 – 4.3], WBDC 103 [4.5 – 3.9]) and should be further investigated along with WBDC accession 021.

All the accessions that were re-tested did not differ significantly from the controls for one or more of the growth parameters measured in the first screening with the exception of WBDC 300 and 345. However, in the second screening, the difference between inoculated treatment and non-inoculated control was significant at $P < 0.0001$ for all the growth parameters measured.

The objective of this study was to identify wild barley accessions that could serve as potential gene donors for resistance to Rhizoctonia root rot in cultivated barley. A wide range of disease reactions were observed among the wild barley diversity collection accession evaluated in this study with only a few accessions in the resistant levels. This was expected, since resistance to *Rhizoctonia* has not been found in cultivated barley to date. Within this study, higher disease ratings were observed in the second screening of potentially resistant accessions compared to the first screening. A possible explanation for the differences in results from the first and second screening might be the differences in the inoculum used. Although the same inoculum density (100 ppg) was used in both experiments, and freshly prepared oat inoculum was specifically used for each screening, this does not overrule the possibility of inherent differences in the aggressiveness or virulence of the inoculum used in both experiments. Also variable distribution of the inoculum from cone to cone could have occurred. However, a similar disease rating was observed for the susceptible check Lenetah in the first (7.4) and second (7.1) experiment. Therefore, the single accession (WBDC 021) indeed exhibits a significant level of resistance and could serve as a possible gene donor for resistance to Rhizoctonia root rot in cultivated barley. Complete resistance to *R. solani* AG-8 has not been identified in other cereals, although moderate levels of resistance were detected in *Dasypyrum villosum* (L.) P. Candargy (Smith et al. 2003). However, being a member of the wheat secondary gene pool, there is difficulty in

transferring this resistant trait to cultivated wheat. In addition, Okubara et al (2009) identified EMS wheat mutants exhibiting tolerance to the pathogen. Although accessions identified in this study appear to be only partially resistant, they offers growers very good sources of resistance, since there has been no report of resistance to Rhizoctonia root rot in barley, and given that there is no hindrance to the transfer of resistant genes from wild barley to cultivated barley. Depending upon the genetic control of resistance, several sources of partial resistance could be highly useful for building quantitatively inherited resistance in cultivated barley. While this study sought to identify resistance to *R. solani* AG-8, screening for resistance to *R. oryzae*, another pathogen known to cause root rot of wheat and barley and found with *R. solani* AG-8 in production fields would provide further information on the reaction of the identified resistant accessions to this species. As this experiment was carried out in controlled environments, field testing is an apparent next step in confirming real resistance to *Rhizoctonia* in WBDC accession 021 several other accessions noted above.

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CONCLUSIONS AND FUTURE RECOMMENDATIONS

To date, resistance to Rhizoctonia root rot caused by *R. solani* AG-8 has not been identified in barley. Every effort in the past to identify resistant barley genotypes has met with little or no success. Although several barley germplasm sources were screened in the past, and some accessions identified as resistant, further testing did not confirm their resistance. In an effort to identify and/or develop resistant barley genotypes that can be used to manage an intractable disease like Rhizoctonia root rot, sodium azide barley mutants, and the Wild Barley (*Hordeum vulgare* subsp. *spontaneum*) Diversity Collection accessions were screened in controlled environments.

From the sodium azide barley mutant screening, 10 putative M_2 individuals were identified and selected from two barley lines, 05WA-316.99 and 'Lenetah'. Further retesting of the progeny of these mutants confirmed resistance in 12 M_3 individuals obtained from both lines. All 12 putative M_3 individuals were backcrossed to the wild-type progenitor for genetic analysis. Reciprocal crosses were carried out to give information as to whether the genes conferring resistance to the pathogen follow a Mendelian or cytoplasmic inheritance pattern. Only BC_1F_1 progeny of 05WA-316.99 were evaluated. Evaluation of BC_1F_1 progeny of Lenetah was not carried out due to partial sterility observed with the mutants of this cultivar.

A wide range of disease reaction was observed among the BC_1F_1 seeds that were evaluated. A greater number of the plants were susceptible to the fungus with only a few plants showing resistant to moderate resistant disease reaction. Reciprocal cross differences were not observed among the BC_1F_1 seeds that were screened. The presence of BC_1F_1 plants displaying resistance to the pathogen is an indication that resistance is not inherited as a recessive trait. Also the greater number of susceptible plants observed suggests the absence of complete dominance. However, these observations were made

on relatively few seeds. In order to gain a better understanding of how the resistance observed is inherited, screening the F_2 progeny of the BC_1F_1 plants would need to be carried out. Observed segregation ratios should be tested against several gene models of inheritance, as it is possible that resistance is conferred by one or more genes, since a sharp division of plants into groups of either resistant and susceptible plants was not observed in the BC_1F_1. Another important step that needs to be carried out is allele tests to determine if the resistance observed in different mutant plants is due to the same or different genes.

From the entire 317 wild barley accessions that were screened, a single accession WBDC 021 displayed consistent moderate resistance to *Rhizoctonia* on the basis of the growth parameters measured in the first and subsequent verification screening experiments. Other accessions that were observed to be moderately resistant in the first screening did not appear resistant in the second screening with the exception of WBDC 005. These two accessions definitely show some potential as a source of Rhizoctonia root rot resistance that can be deployed for use in cultivated barley. Since these accessions were evaluated in controlled environments, it is important to test these accessions in the field to determine if the resistance observed in the greenhouse can be replicated in the field. Since the accessions of the Wild Barley Diversity Collection have been genotyped with an array of molecular markers, another major step is to identify and map specific genes or QTLs involved in the resistant reaction.

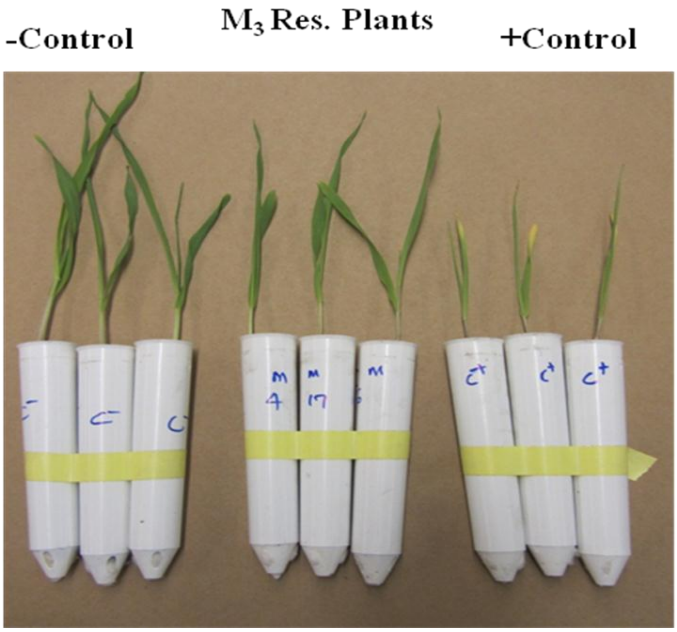

Figure 1: Three of the putative M_3 mutants of breeding line 05WA316.99 compared with its wild-type progenitor in the presence and absence of Rhizoctonia inoculum

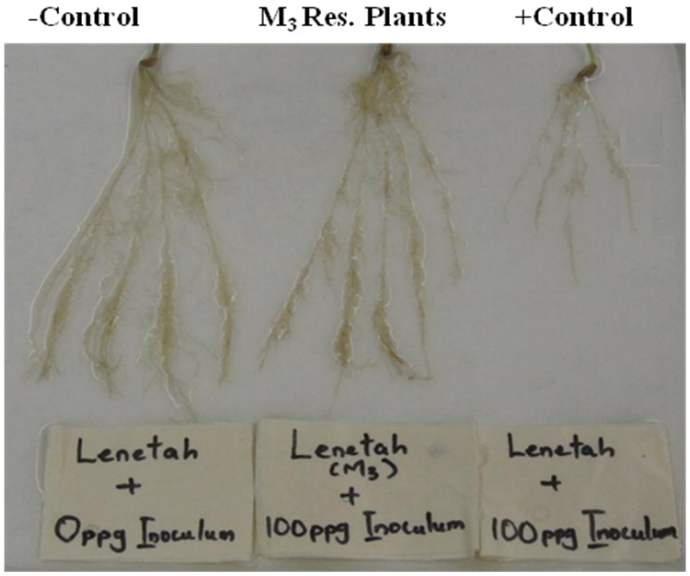

Figure 2: Root of a putative M_3 mutant of cultivar Lenetah compared with roots of its wild-type progenitor in the presence and absence of Rhizoctonia inoculum

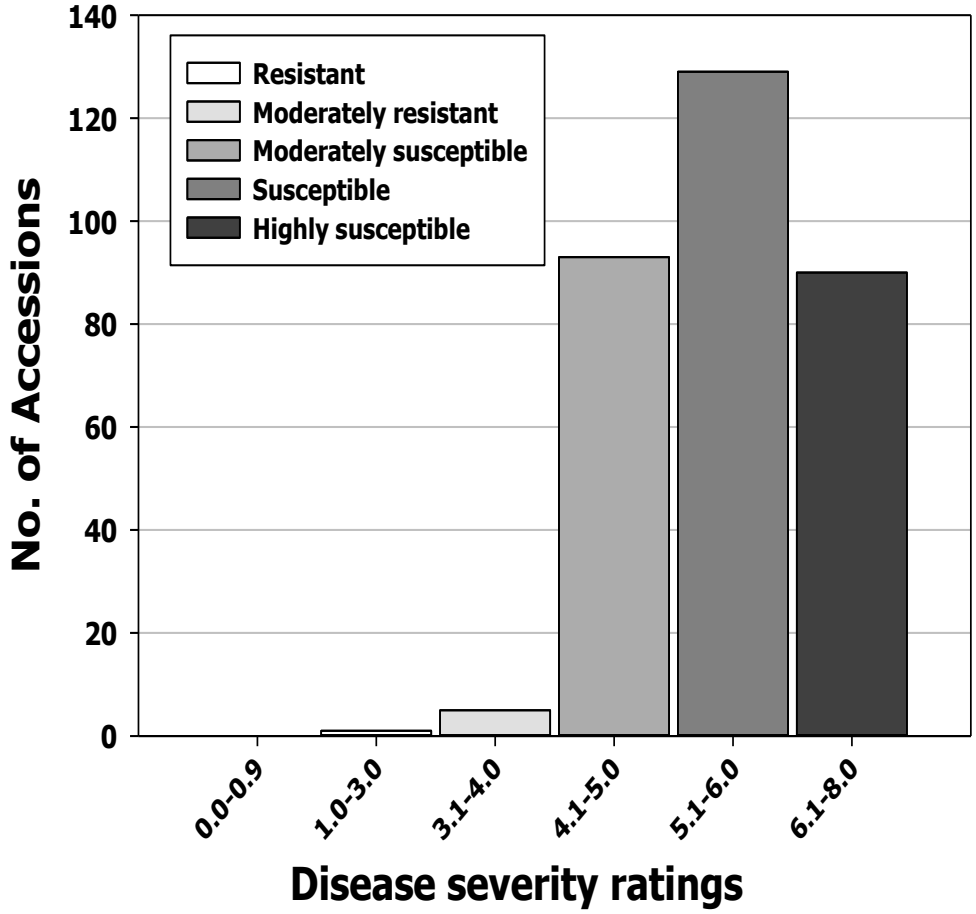

Figure 3: Distribution of 317 wild barley accession based on Rhizoctonia root rot disease severity ratings

Table 1: Distribution of origin of 317 accessions of the Wild Barley Diversity Collection (*Hordeum vulgare* subsp. *spontaneum*)

Country	**Number of Accessions**
Afghanistan	7
Armenia	1
Azerbaijan	7
Cyprus	3
Egypt	1
Iran	18
Iraq	10
Israel	3
Jordan	61
Kazakhstan	3
Lebanon	19
Libya	8
Pakistan	2
Palestine	38
Russia	1
Syria	72
Tajikistan	7
Turkey	23
Turkmenistan	18
Uzbekistan	13
Unknown	2
Total	**317**

Table 2: Accession, *Rhizoctonia solani* AG-8 and accession x Rhizoctonia effects on response variables measured in initial screening of the 317 accessions.

Response variable	Accession		*R. solani* AG-8		Interaction	
	F value	P value	F value	P value	F value	P value
Disease rating	9.91	< 0.0001	51031.6	< 0.0001	9.91	< 0.0001
Shoot length	23.25	< 0.0001	3954.54	< 0.0001	5.85	< 0.0001
Seedling weight	14.33	< 0.0001	3747.2	< 0.0001	4.36	< 0.0001
Root weight	14.79	< 0.0001	2133.69	< 0.0001	8.33	< 0.0001

Table 3: Comparison of estimates of differences between the *R. solani* AG-8 inoculated treatment and non-inoculated control for shoot length, root weight and seedling weight of resistant (1), moderately resistant (5), and moderately susceptible (5) accessions that showed potential for some level of resistance.

Accession	Experiment 1			Experiment 2		
	Compared with control			Compared with control		
	Diff[†]	SE[§]	P value	Diff[†]	SE[§]	P value
Shoot length (cm)						
WBDC 021	2.26	1.359	**0.0958**[*]	9.37	1.363	<.0001
WBDC 013	2.59	1.359	**0.0570**[*]	13.52	1.363	<.0001
WBDC 345	7.06	1.403	<.0001	8.15	1.400	<.0001
WBDC 005	4.69	1.531	0.0022	4.89	1.481	0.0011
WBDC 300	3.46	1.559	0.0265	12.21	1.400	<.0001
WBDC 007	4.38	1.323	0.0009	9.94	1.502	<.0001
WBDC 080	2.93	1.205	**0.1998**[*]	12.73	1.400	<.0001
WBDC 009	3.99	1.359	0.0034	8.26	1.400	<.0001
WBDC 079	3.37	1.278	**0.6844**[*]	13.42	1.481	<.0001
WBDC 085	2.01	1.205	**0.3008**[*]	10.27	1.363	<.0001
WBDC 103	8.66	1.403	<.0001	9.42	1.400	<.0001
Root weight (g)						
WBDC 021	0.309	0.039	**0.3492**[*]	0.170	0.025	<.0001
WBDC 013	0.359	0.039	**0.0572**[*]	0.173	0.025	<.0001
WBDC 345	0.430	0.041	0.0002	0.187	0.025	<.0001
WBDC 005	0.370	0.044	**0.0548**[*]	0.124	0.027	<.0001
WBDC 300	0.359	0.045	0.0011	0.267	0.025	<.0001
WBDC 007	0.119	0.038	**0.6851**[*]	0.127	0.027	<.0001
WBDC 080	0.099	0.034	0.0234	0.285	0.025	<.0001
WBDC 009	0.102	0.039	**0.9348**[*]	0.203	0.025	<.0001
WBDC 079	0.029	0.036	0.0012	0.180	0.027	<.0001
WBDC 085	0.083	0.034	0.0402	0.175	0.025	<.0001
WBDC 103	0.100	0.041	0.0007	0.151	0.025	<.0001

[†] Estimates of differences between least square means of inoculum treatments and non-inoculated controls

[§]Standard error of the differences

[*]Differences between inoculum treatment and non-inoculated control are not significant at $P < 0.05$

Table 3 Continued…

Accession	Experiment 1			Experiment 2		
	Compared with control			Compared with control		
	Diff[†]	SE[§]	P value	Diff[†]	SE[§]	P value
Seedling weight (g)						
WBDC 021	0.056	0.045	0.0068	0.237	0.028	<.0001
WBDC 013	0.100	0.045	0.0004	0.271	0.028	<.0001
WBDC 345	0.164	0.046	<.0001	0.204	0.029	<.0001
WBDC 005	0.117	0.050	0.003	0.128	0.030	<.0001
WBDC 300	0.127	0.051	<.0001	0.292	0.029	<.0001
WBDC 007	-0.016	0.043	**0.142**[*]	0.215	0.031	<.0001
WBDC 080	-0.081	0.043	0.0164	0.336	0.029	<.0001
WBDC 009	-0.004	0.045	**0.3068**[*]	0.220	0.029	<.0001
WBDC 079	-0.104	0.046	0.0115	0.248	0.030	<.0001
WBDC 085	-0.072	0.043	**0.1025**[*]	0.244	0.028	<.0001
WBDC 103	-0.108	0.046	**0.1314**[*]	0.186	0.029	<.0001

[†] Estimates of differences between least square means of inoculum treatments and non-inoculated controls

[§]Standard error of the differences

[*]Differences between inoculum treatment and non-inoculated control are not significant at $P < 0.05$

Table 4: Correlations between disease severity ratings, shoot length, seedling weight and root weight of wild barley accessions screened for resistance to Rhizoctonia root rot.

Response variable	Correlation coefficient*		
	Seedling weight	Root weight	Shoot length
Expt. 1			
Disease rating	-0.71	-0.58	-0.67
Shoot length	0.64	0.50	
Root wt.	0.93		
Expt. 2			
Disease rating	-0.89	-0.86	-0.90
Shoot length	0.93	0.86	
Root wt.	0.96		

* All correlation coefficient values are significant at $P < 0.0001$. Experiment 1= First screening of the entire 317 accessions. Experiment 2: Re-testing of 11 promising accessions selected from first screening.

Table 5: Accession, *Rhizoctonia solani* AG-8 and accession x Rhizoctonia effects on response variables measured in second screening of 11 promising accessions selected from the initial screening and 3 checks

Response variable	Accession		*R. solani* AG-8		Interaction	
	F value	P value	F value	P value	F value	P value
Disease rating	6.48	< 0.0001	3577.58	< 0.0001	6.48	< 0.0001
Shoot length	2.88	< 0.0001	835.34	< 0.0001	3.50	< 0.0001
Seedling weight	8.87	< 0.0001	1051.07	< 0.0001	3.78	< 0.0001
Root weight	8.46	< 0.0001	878.43	< 0.0001	5.08	< 0.0001

Table 6: Rhizoctonia root rot disease ratings of 11 promising accessions in first and second screening

Group	Accessions	Expt. 1[¶]	Expt. 2[¶]
		Disease rating	Disease rating
Res. [*]	WBDC 021	1.6 ± 0.202	3.6 ± 0.208
Mod.Res. [*]	WBDC 013	3.4 ± 0.202	5.1 ± 0.208
	WBDC 345	3.6 ± 0.191	4.6 ± 0.219
	WBDC 005	3.7 ± 0.229	4.0 ± 0.232
	WBDC 300	3.8 ± 0.247	4.6 ± 0.208
	WBDC 007	3.9 ± 0.191	5.1 ± 0.248
Mod.Sus. [*]	WBDC 080	4.2 ± 0.188	5.2 ± 0.219
	WBDC 009	4.2 ± 0.202	4.3 ± 0.219
	WBDC 079	4.3 ± 0.210	5.4 ± 0.219
	WBDC 085	4.4 ± 0.188	4.3 ± 0.208
	WBDC 103	4.5 ± 0.214	3.9 ± 0.219
Check	Lenetah	7.4 ± 0.229	7.1 ± 0.219
	Bob		5.7 ± 0.208
	05WA-316.99		5.7 ± 0.208

[¶] Least square means of Rhizoctonia root rot disease severity ratings and standard errors

[*] Res = Resistant, Mod. Res. = Moderately resistant, Mod. Sus. = Moderately susceptible.

Appendix 1: Disease severity ratings of 317 wild barley accessions in initial screening for resistance to Rhizoctonia root rot

Accession	IG#*	Origin	DR¶	SE§
Lenetah	Cultivated Barley	USA	7.4	0.229
WBDC001	38610	SYR	4.3	0.191
WBDC002	38611	SYR	4.9	0.202
WBDC004	38614	SYR	5.2	0.202
WBDC005	38616	JOR	3.7	0.229
WBDC006	38619	JOR	5.3	0.202
WBDC007	38623	JOR	3.9	0.191
WBDC008	38627	JOR	5.7	0.202
WBDC009	38633	JOR	4.2	0.202
WBDC010	38653	AFG	4.4	0.202
WBDC011	38655	IRQ	5.0	0.191
WBDC012	38656	AFG	6.0	0.214
WBDC013	38658	IRQ	3.4	0.202
WBDC014	38659	AFG	5.8	0.214
WBDC015	38660	AFG	4.9	0.202
WBDC016	38661	IRN	4.8	0.202
WBDC017	38665	SYR	4.3	0.214
WBDC018	38670	AFG	5.4	0.191
WBDC019	38671	IRN	4.7	0.202
WBDC020	38672	TUR	5.0	0.191
WBDC021	38678	IRQ	1.6	0.202
WBDC022	38679	TUR	4.3	0.188
WBDC023	38681	IRN	4.5	0.188
WBDC024	38682	IRN	6.6	0.188
WBDC025	38693	PAK	6.2	0.188
WBDC026	38826	TJK	4.6	0.198
WBDC027	38828	AZE	4.8	0.188
WBDC028	38840	PAL	5.3	0.188
WBDC029	38843	PAL	5.1	0.188
WBDC030	38853	PAL	6.5	0.188
WBDC031	38860	PAL	4.8	0.198
WBDC032	38869	PAL	6.2	0.188
WBDC033	38886	PAL	5.1	0.188
WBDC034	38912	PAL	5.7	0.188
WBDC035	38981	PAL	6.1	0.188
WBDC036	39002	AFG	5.9	0.188
WBDC037	39082	PAL	5.6	0.188

WBDC038	39117	PAL	5.3	0.188
WBDC039	39393	JOR	5.4	0.210
WBDC040	39591	PAL	6.0	0.188
WBDC041	39603	PAL	5.6	0.198
WBDC042	39673	PAL	5.3	0.188
WBDC043	39704	PAL	5.2	0.243
WBDC044	39759	PAL	4.2	0.198
WBDC045	39821	JOR	4.6	0.188
WBDC046	39824	JOR	4.5	0.188
WBDC047	39825	JOR	5.4	0.266
WBDC048	39837	TUR	4.4	0.188
WBDC049	39839	TUR	5.5	0.188
WBDC050	39846	SYR	5.1	0.210
WBDC051	39847	SYR	4.8	0.243
WBDC052	39850	JOR	4.3	0.188
WBDC053	39852	PAK	5.1	0.198
WBDC054	39857	SYR	5.3	0.198
WBDC055	39859	SYR	5.2	0.188
WBDC056	39876	TUR	5.9	0.225
WBDC057	39880	SYR	5.4	0.225
WBDC058	39885	CYP	5.0	0.210
WBDC059	39886	CYP	4.6	0.198
WBDC060	39891	EGY	4.7	0.198
WBDC061	39910	SYR	5.0	0.198
WBDC062	39911	SYR	5.8	0.188
WBDC063	39913	SYR	5.2	0.188
WBDC064	39914	SYR	5.9	0.188
WBDC065	39915	SYR	5.4	0.188
WBDC066	39916	SYR	5.3	0.210
WBDC067	39917	SYR	5.7	0.188
WBDC068	39918	SYR	4.6	0.198
WBDC069	39920	SYR	4.4	0.198
WBDC070	39932	SYR	5.6	0.198
WBDC072	39935	LBY	4.7	0.198
WBDC073	39936	LBY	5.2	0.188
WBDC074	39937	LBY	4.8	0.188
WBDC075	39939	LBY	5.4	0.188
WBDC078	39942	SYR	5.4	0.225
WBDC079	39990	JOR	4.3	0.210
WBDC080	39996	JOR	4.2	0.188
WBDC081	40002	JOR	5.1	0.188

WBDC082	40009	JOR	5.4	0.188
WBDC083	40012	JOR	5.6	0.188
WBDC085	40015	JOR	4.4	0.188
WBDC089	40026	JOR	5.5	0.247
WBDC092	40034	JOR	5.0	0.214
WBDC093	40035	JOR	5.3	0.247
WBDC094	40039	JOR	5.4	0.229
WBDC095	40045	JOR	5.1	0.214
WBDC097	40051	JOR	5.0	0.229
WBDC100	40060	JOR	6.1	0.214
WBDC101	40063	JOR	5.1	0.214
WBDC102	40064	JOR	6.3	0.202
WBDC103	40071	JOR	4.5	0.214
WBDC104	40072	JOR	5.3	0.229
WBDC105	40075	JOR	6.3	0.303
WBDC106	40077	SYR	6.2	0.191
WBDC107	40078	SYR	5.3	0.247
WBDC108	40080	SYR	7.4	0.229
WBDC109	40090	SYR	4.5	0.247
WBDC110	40091	SYR	5.7	0.229
WBDC111	40097	SYR	5.0	0.229
WBDC112	40098	SYR	5.4	0.214
WBDC113	40099	TKM	5.5	0.214
WBDC115	40104	TKM	5.0	0.214
WBDC116	40105	TKM	4.9	0.229
WBDC117	40106	TKM	5.1	0.214
WBDC119	40108	UZB	6.0	0.247
WBDC120	40109	TJK	5.1	0.191
WBDC121	40138	IRN	5.5	0.214
WBDC122	40140	IRN	6.0	0.202
WBDC123	40142	IRN	5.8	0.247
WBDC124	40143	IRN	6.5	0.247
WBDC125	40154	UZB	5.3	0.247
WBDC126	40156	LBN	4.6	0.188
WBDC127	40159	SYR	5.1	0.210
WBDC128	40164	SYR	5.0	0.210
WBDC129	40171	SYR	5.2	0.243
WBDC130	40173	SYR	4.3	0.210
WBDC131	40174	SYR	4.3	0.243
WBDC132	40177	LBN	5.2	0.198
WBDC133	40178	LBN	5.8	0.188

WBDC134	40179	LBN	4.6	0.198
WBDC135	40180	LBN	5.8	0.188
WBDC136	40181	LBN	4.4	0.198
WBDC137	40182	LBN	5.6	0.225
WBDC138	40183	LBN	5.0	0.188
WBDC139	40184	LBN	4.8	0.198
WBDC140	40186	LBN	4.8	0.210
WBDC141	40187	LBN	5.1	0.198
WBDC142	40188	LBN	4.9	0.198
WBDC143	40191	LBN	4.6	0.198
WBDC145	40194	LBN	5.1	0.210
WBDC146	40196	IRN	5.9	0.225
WBDC147	40197	IRN	6.3	0.225
WBDC148	40198	IRN	6.3	0.243
WBDC149	40199	IRN	6.0	0.266
WBDC150	40200	IRN	7.1	0.225
WBDC151	40201	SYR	6.4	0.210
WBDC152	107046	IRN	6.6	0.225
WBDC153	107047	IRN	7.4	0.210
WBDC154	107423	IRQ	6.3	0.243
WBDC155	107424	IRQ	6.5	0.243
WBDC156	107425	IRQ	4.8	0.188
WBDC157	107426	IRQ	6.8	0.266
WBDC158	107427	IRQ	6.7	0.243
WBDC159	110739	SYR	6.7	0.243
WBDC160	110742	SYR	6.0	0.225
WBDC161	110751	SYR	6.4	0.210
WBDC164	110773	SYR	6.8	0.266
WBDC165	110793	SYR	6.0	0.210
WBDC166	110798	SYR	7.0	0.243
WBDC167	110804	SYR	6.8	0.210
WBDC168	110816	LBN	6.3	0.225
WBDC169	110819	LBN	6.7	0.225
WBDC170	110831	LBN	6.0	0.210
WBDC171	110833	LBN	5.8	0.243
WBDC172	112673	IRN	7.0	0.266
WBDC173	112674	IRN	5.4	0.198
WBDC174	112679	IRN	6.0	0.225
WBDC177	112797	IRQ	7.6	0.225
WBDC178	112813	IRQ	6.2	0.243
WBDC179	112846	LBY	5.6	0.225

WBDC180	112847	LBY	5.7	0.225
WBDC181	115780	JOR	6.0	0.191
WBDC182	115781	JOR	4.7	0.191
WBDC183	115789	JOR	4.4	0.202
WBDC184	116004	LBY	4.8	0.191
WBDC185	116005	LBY	4.6	0.214
WBDC186	116099	TUR	5.4	0.229
WBDC187	116100	TUR	6.0	0.247
WBDC188	116104	TUR	5.2	0.202
WBDC189	116105	TUR	5.6	0.214
WBDC190	116106	TUR	5.2	0.202
WBDC191	116107	TUR	5.3	0.202
WBDC192	116108	TUR	5.9	0.214
WBDC193	116118	TUR	5.5	0.191
WBDC194	116119	TUR	4.6	0.229
WBDC195	116121	TUR	5.5	0.303
WBDC196	116125	TUR	6.0	0.191
WBDC197	119386	SYR	6.1	0.214
WBDC198	119402	SYR	5.9	0.191
WBDC199	119424	SYR	4.1	0.229
WBDC200	119427	SYR	5.3	0.202
WBDC201	119443	SYR	5.4	0.214
WBDC202	119451	SYR	5.3	0.191
WBDC203	119458	SYR	5.5	0.191
WBDC204	120794	TKM	4.7	0.202
WBDC205	120920	RUS	4.1	0.202
WBDC206	121857	SYR	5.4	0.229
WBDC207	123949	UZB	5.0	0.202
WBDC208	123959	UZB	6.8	0.303
WBDC209	123972	UZB	5.6	0.202
WBDC210	123991	UZB	5.0	0.202
WBDC211	124000	UZB	6.8	0.191
WBDC212	124017	UZB	6.1	0.229
WBDC213	124035	UZB	7.0	0.428
WBDC214	124046	UZB	7.4	0.271
WBDC215	126427	TKM	5.4	0.214
WBDC216	126484	TKM	7.0	0.605
WBDC217	126933	ARM	5.2	0.271
WBDC218	131375	KAZ	7.0	0.605
WBDC219	131620	KAZ	6.8	0.247
WBDC220	131642	KAZ	6.5	0.247

WBDC221	131674	TJK	6.0	0.349
WBDC222	131675	TJK	6.3	0.303
WBDC223	131684	TJK	6.4	0.271
WBDC224	131790	TJK	6.3	0.247
WBDC225	131792	TJK	7.0	0.303
WBDC227	132552	AZE	6.0	0.349
WBDC228	132606	AZE	6.8	0.271
WBDC229	132623	AZE	6.5	0.303
WBDC230	132627	AZE	6.0	0.428
WBDC231	132636	AZE	5.9	0.229
WBDC232	132666	AZE	4.6	0.188
WBDC233	38668	AFG	5.3	0.225
WBDC234	39884	CYP	5.7	0.198
WBDC235	40010	JOR	7.3	0.210
WBDC236	40029	JOR	6.3	0.225
WBDC237	40033	JOR	5.8	0.243
WBDC238	40037	JOR	6.8	0.266
WBDC240	135267	JOR	6.0	0.210
WBDC241	135273	JOR	6.8	0.297
WBDC242	38620	JOR	5.3	0.243
WBDC243	40000	JOR	6.3	0.210
WBDC244	135700	JOR	6.3	0.198
WBDC245	38621	JOR	6.0	0.297
WBDC246	38626	JOR	5.8	0.188
WBDC247	38630	JOR	6.8	0.210
WBDC248	39394	JOR	5.3	0.343
WBDC250	39988	JOR	6.8	0.210
WBDC252	40008	JOR	5.2	0.198
WBDC253	115787	JOR	5.1	0.188
WBDC254	115790	JOR	6.4	0.198
WBDC255	115792	JOR	7.4	0.266
WBDC256	135856	JOR	5.5	0.243
WBDC257	39399	JOR	6.1	0.210
WBDC258	40041	JOR	6.7	0.343
WBDC259	40047	JOR	7.1	0.210
WBDC260	39822	JOR	5.8	0.243
WBDC261	40065	JOR	6.6	0.188
WBDC262	40069	JOR	5.1	0.225
WBDC263	40070	JOR	6.1	0.225
WBDC265	40052	JOR	6.1	0.225
WBDC266	40054	JOR	6.4	0.210

WBDC267	40062	JOR	5.1	0.210
WBDC268	135254	JOR	7.0	0.297
WBDC269	39546	LBN	6.0	0.225
WBDC270	38866	PAL	5.9	0.225
WBDC271	38938	PAL	5.7	0.243
WBDC274	39387	PAL	7.4	0.266
WBDC275	39390	PAL	6.3	0.198
WBDC276	38862	PAL	7.0	0.210
WBDC277	38942	PAL	6.4	0.225
WBDC278	39108	PAL	4.8	0.214
WBDC279	39386	PAL	5.6	0.214
WBDC280	39687	PAL	5.8	0.247
WBDC281	38832	PAL	5.3	0.202
WBDC282	38874	PAL	4.4	0.214
WBDC283	38880	PAL	5.4	0.229
WBDC284	38939	PAL	5.3	0.214
WBDC285	39071	PAL	5.5	0.247
WBDC286	39080	PAL	5.1	0.229
WBDC287	39092	PAL	6.0	0.202
WBDC288	39700	PAL	5.1	0.214
WBDC289	39714	PAL	5.0	0.247
WBDC290	38891	PAL	6.0	0.271
WBDC291	38906	PAL	4.8	0.214
WBDC292	38926	PAL	6.2	0.247
WBDC293	38932	PAL	7.3	0.303
WBDC294	38950	PAL	5.0	0.271
WBDC295	110780	SYR	4.5	0.303
WBDC296	135336	SYR	4.7	0.229
WBDC297	135346	SYR	5.6	0.229
WBDC298	135357	SYR	5.0	0.303
WBDC299	39912	SYR	5.0	0.202
WBDC300	117896	SYR	3.8	0.247
WBDC302	38635	SYR	4.6	0.214
WBDC303	38640	SYR	4.9	0.191
WBDC304	40161	SYR	5.0	0.202
WBDC305	40162	SYR	4.8	0.214
WBDC306	40166	SYR	6.3	0.202
WBDC307	40175	SYR	4.5	0.247
WBDC308	40082	SYR	5.7	0.229
WBDC309	119435	SYR	8.0	0.349
WBDC310	39849	SYR	8.0	0.229

WBDC311	39882	SYR	7.0	0.229
WBDC312	40079	SYR	8.0	0.229
WBDC314	119420	SYR	8.0	0.247
WBDC315	119431	SYR	7.7	0.247
WBDC316	38613	SYR	7.6	0.202
WBDC317	39843	SYR	6.8	0.247
WBDC318	39919	SYR	7.5	0.247
WBDC319	40084	SYR	8.0	0.247
WBDC320	40094	SYR	7.9	0.214
WBDC323	135606	TKM	7.0	0.303
WBDC324	135609	TKM	8.0	0.247
WBDC326	38813	TKM	7.4	0.229
WBDC329	135537	TKM	8.0	0.271
WBDC330	135504	TKM	7.4	0.271
WBDC331	135507	TKM	7.6	0.271
WBDC332	135460	TKM	4.1	0.229
WBDC333	135478	TKM	4.1	0.202
WBDC334	135563	TKM	4.6	0.202
WBDC335	135624	TKM	5.1	0.202
WBDC336	126406	TKM	4.7	0.202
WBDC337	116111	TUR	5.0	0.202
WBDC338	116112	TUR	4.8	0.202
WBDC340	116116	TUR	4.1	0.229
WBDC341	116126	TUR	5.0	0.202
WBDC342	116128	TUR	4.8	0.247
WBDC343	116130	TUR	4.2	0.202
WBDC344	38674	TUR	4.7	0.202
WBDC345	40155	UZB	3.6	0.191
WBDC346	120795	UZB	4.2	0.202
WBDC347	40152	UZB	5.0	0.202
WBDC348	Damon 11-11 (B)	ISR	5.1	0.229
WBDC349	Shechem 12-32 (A)	ISR	5.3	0.214
WBDC350	41-1 (#1)	ISR	4.3	0.191
WBDC354	HS 680		4.7	0.191
WBDC355	OUH602		4.5	0.214

* Unique gene bank identification number assigned for accession identification

¶ Disease severity ratings

§ Standard error

Appendix 2: Comparison of estimates of differences between inoculated treatment and non-inoculated control for shoot length of 317 wild barley accessions in first screening for Rhizoctonia root rot resistance.

Accession	IG #	Origin	Diff†	SE§	P value
Lenetah	Cultivated Barley	USA	17.22	1.491	<.0001
WBDC001	38610	SYR	6.74	1.323	<.0001
WBDC002	38611	SYR	4.98	1.359	0.0003
WBDC004	38614	SYR	7.31	1.394	<.0001
WBDC005	38616	JOR	4.69	1.531	0.0022
WBDC006	38619	JOR	4.06	1.437	0.0048
WBDC007	38623	JOR	4.38	1.323	0.0009
WBDC008	38627	JOR	7.44	1.359	<.0001
WBDC009	38633	JOR	3.99	1.359	0.0034
WBDC010	38653	AFG	3.62	1.359	0.0077
WBDC011	38655	IRQ	2.28	1.323	**0.0849***
WBDC012	38656	AFG	7.48	1.403	<.0001
WBDC013	38658	IRQ	2.59	1.359	**0.0570***
WBDC014	38659	AFG	3.09	1.531	0.0434
WBDC015	38660	AFG	9.44	1.359	<.0001
WBDC016	38661	IRN	1.94	1.394	**0.1633***
WBDC017	38665	SYR	6.33	1.403	<.0001
WBDC018	38670	AFG	4.98	1.323	0.0002
WBDC019	38671	IRN	9.31	1.359	<.0001
WBDC020	38672	TUR	9.64	1.323	<.0001
WBDC021	38678	IRQ	2.26	1.359	**0.0958***
WBDC022	38679	TUR	5.42	1.238	0.0013
WBDC023	38681	IRN	3.53	1.238	0.0027
WBDC024	38682	IRN	3.95	1.205	<.0001
WBDC025	38693	PAK	5.10	1.205	0.0034
WBDC026	38826	TJK	2.12	1.238	0.001
WBDC027	38828	AZE	4.28	1.205	<.0001
WBDC028	38840	PAL	11.48	1.205	<.0001
WBDC029	38843	PAL	4.77	1.205	<.0001
WBDC030	38853	PAL	6.05	1.205	0.0006
WBDC031	38860	PAL	4.15	1.238	0.0009
WBDC032	38869	PAL	6.06	1.238	<.0001
WBDC033	38886	PAL	4.40	1.238	0.0001
WBDC034	38912	PAL	4.40	1.205	0.0027
WBDC035	38981	PAL	6.59	1.205	<.0001
WBDC036	39002	AFG	4.44	1.238	0.0011

WBDC037	39082	PAL	6.58	1.238	<.0001
WBDC038	39117	PAL	6.00	1.238	<.0001
WBDC039	39393	JOR	3.44	1.394	<.0001
WBDC040	39591	PAL	5.92	1.391	<.0001
WBDC041	39603	PAL	3.78	1.238	**0.0556***
WBDC042	39673	PAL	7.09	1.238	<.0001
WBDC043	39704	PAL	6.75	1.391	<.0001
WBDC044	39759	PAL	2.27	1.238	<.0001
WBDC045	39821	JOR	5.42	1.205	<.0001
WBDC046	39824	JOR	1.78	1.238	<.0001
WBDC047	39825	JOR	6.73	1.535	<.0001
WBDC048	39837	TUR	3.66	1.205	<.0001
WBDC049	39839	TUR	5.79	1.238	<.0001
WBDC050	39846	SYR	4.65	1.347	<.0001
WBDC051	39847	SYR	5.67	1.455	<.0001
WBDC052	39850	JOR	3.87	1.238	<.0001
WBDC053	39852	PAK	6.96	1.238	<.0001
WBDC054	39857	SYR	5.16	1.238	<.0001
WBDC055	39859	SYR	4.78	1.205	<.0001
WBDC056	39876	TUR	6.10	1.394	<.0001
WBDC057	39880	SYR	7.61	1.498	<.0001
WBDC058	39885	CYP	5.55	1.309	<.0001
WBDC059	39886	CYP	2.20	1.270	<.0001
WBDC060	39891	EGY	5.28	1.238	<.0001
WBDC061	39910	SYR	4.28	1.270	<.0001
WBDC062	39911	SYR	7.73	1.205	0.0027
WBDC063	39913	SYR	1.99	1.205	0.0233
WBDC064	39914	SYR	7.84	1.278	<.0001
WBDC065	39915	SYR	1.43	1.205	**0.9573***
WBDC066	39916	SYR	6.00	1.278	**0.0557***
WBDC067	39917	SYR	4.70	1.238	0.0028
WBDC068	39918	SYR	5.39	1.309	0.0432
WBDC069	39920	SYR	1.63	1.238	**0.9341***
WBDC070	39932	SYR	4.58	1.309	**0.2235***
WBDC072	39935	LBY	2.56	1.238	**0.2069***
WBDC073	39936	LBY	1.94	1.205	**0.4510***
WBDC074	39937	LBY	4.83	1.238	**0.8732***
WBDC075	39939	LBY	3.31	1.205	<.0001
WBDC078	39942	SYR	3.23	1.440	0.009
WBDC079	39990	JOR	3.37	1.278	**0.6844***
WBDC080	39996	JOR	2.93	1.205	**0.1998***

WBDC081	40002	JOR	7.86	1.205	0.0001
WBDC082	40009	JOR	7.41	1.205	0.0012
WBDC083	40012	JOR	3.47	1.205	**0.0945***
WBDC085	40015	JOR	2.01	1.205	**0.3008***
WBDC089	40026	JOR	6.07	1.559	0.0001
WBDC092	40034	JOR	8.49	1.403	<.0001
WBDC093	40035	JOR	10.61	1.559	<.0001
WBDC094	40039	JOR	8.54	1.491	<.0001
WBDC095	40045	JOR	3.53	1.403	0.0119
WBDC097	40051	JOR	11.59	1.581	<.0001
WBDC100	40060	JOR	9.48	1.437	<.0001
WBDC101	40063	JOR	10.61	1.437	<.0001
WBDC102	40064	JOR	8.38	1.491	<.0001
WBDC103	40071	JOR	8.66	1.403	<.0001
WBDC104	40072	JOR	9.02	1.491	<.0001
WBDC105	40075	JOR	7.89	1.750	<.0001
WBDC106	40077	SYR	6.55	1.323	<.0001
WBDC107	40078	SYR	6.46	1.646	<.0001
WBDC108	40080	SYR	8.37	1.531	<.0001
WBDC109	40090	SYR	6.15	1.559	<.0001
WBDC110	40091	SYR	9.54	1.531	<.0001
WBDC111	40097	SYR	5.70	1.458	<.0001
WBDC112	40098	SYR	8.39	1.479	<.0001
WBDC113	40099	TKM	4.79	1.479	0.0012
WBDC115	40104	TKM	5.19	1.437	0.0003
WBDC116	40105	TKM	7.50	1.491	<.0001
WBDC117	40106	TKM	12.57	1.437	<.0001
WBDC119	40108	UZB	8.28	1.598	<.0001
WBDC120	40109	TJK	7.57	1.323	<.0001
WBDC121	40138	IRN	7.96	1.403	<.0001
WBDC122	40140	IRN	11.13	1.359	<.0001
WBDC123	40142	IRN	6.59	1.559	<.0001
WBDC124	40143	IRN	9.86	1.528	<.0001
WBDC125	40154	UZB	9.56	1.559	<.0001
WBDC126	40156	LBN	5.58	1.238	<.0001
WBDC127	40159	SYR	7.41	1.347	<.0001
WBDC128	40164	SYR	6.58	1.309	<.0001
WBDC129	40171	SYR	7.53	1.905	<.0001
WBDC130	40173	SYR	5.53	1.278	<.0001
WBDC131	40174	SYR	7.18	1.391	<.0001
WBDC132	40177	LBN	7.46	1.357	<.0001

WBDC133	40178	LBN	7.74	1.278	<.0001
WBDC134	40179	LBN	5.04	1.502	<.0001
WBDC135	40180	LBN	6.59	1.278	<.0001
WBDC136	40181	LBN	6.73	1.238	<.0001
WBDC137	40182	LBN	8.67	1.440	<.0001
WBDC138	40183	LBN	4.06	1.278	0.001
WBDC139	40184	LBN	5.57	1.238	<.0001
WBDC140	40186	LBN	4.62	1.278	<.0001
WBDC141	40187	LBN	6.02	1.238	<.0001
WBDC142	40188	LBN	6.52	1.309	<.0001
WBDC143	40191	LBN	5.70	1.238	<.0001
WBDC145	40194	LBN	6.86	1.278	<.0001
WBDC146	40196	IRN	6.92	1.394	<.0001
WBDC147	40197	IRN	2.10	1.357	<.0001
WBDC148	40198	IRN	5.63	1.555	<.0001
WBDC149	40199	IRN	4.35	1.535	0.0003
WBDC150	40200	IRN	4.61	1.394	<.0001
WBDC151	40201	SYR	5.61	1.309	<.0001
WBDC152	107046	IRN	8.76	1.394	<.0001
WBDC153	107047	IRN	8.95	1.347	<.0001
WBDC154	107423	IRQ	7.25	1.555	<.0001
WBDC155	107424	IRQ	7.95	1.455	<.0001
WBDC156	107425	IRQ	7.69	1.278	<.0001
WBDC157	107426	IRQ	8.77	1.577	<.0001
WBDC158	107427	IRQ	8.61	1.420	<.0001
WBDC159	110739	SYR	7.37	1.555	<.0001
WBDC160	110742	SYR	8.67	1.577	<.0001
WBDC161	110751	SYR	3.91	1.309	0.0002
WBDC164	110773	SYR	9.57	1.535	<.0001
WBDC165	110793	SYR	5.97	1.309	<.0001
WBDC166	110798	SYR	8.36	1.420	<.0001
WBDC167	110804	SYR	7.92	1.455	<.0001
WBDC168	110816	LBN	4.77	1.394	<.0001
WBDC169	110819	LBN	4.49	1.394	<.0001
WBDC170	110831	LBN	6.77	1.309	<.0001
WBDC171	110833	LBN	8.47	1.391	<.0001
WBDC172	112673	IRN	8.44	1.502	<.0001
WBDC173	112674	IRN	5.03	1.270	<.0001
WBDC174	112679	IRN	4.77	1.394	<.0001
WBDC177	112797	IRQ	6.04	1.327	<.0001
WBDC178	112813	IRQ	7.13	1.555	<.0001

WBDC179	112846	LBY	4.03	1.327	<.0001
WBDC180	112847	LBY	3.22	1.357	<.0001
WBDC181	115780	JOR	7.87	1.403	<.0001
WBDC182	115781	JOR	8.86	1.323	<.0001
WBDC183	115789	JOR	4.51	1.359	0.0009
WBDC184	116004	LBY	7.99	1.359	<.0001
WBDC185	116005	LBY	6.54	1.479	<.0001
WBDC186	116099	TUR	10.55	1.458	<.0001
WBDC187	116100	TUR	8.38	1.598	<.0001
WBDC188	116104	TUR	11.03	1.359	<.0001
WBDC189	116105	TUR	9.00	1.437	<.0001
WBDC190	116106	TUR	9.06	1.359	<.0001
WBDC191	116107	TUR	7.74	1.394	<.0001
WBDC192	116108	TUR	9.12	1.403	<.0001
WBDC193	116118	TUR	11.14	1.323	<.0001
WBDC194	116119	TUR	6.49	1.581	<.0001
WBDC195	116121	TUR	4.00	1.984	0.0439
WBDC196	116125	TUR	9.84	1.359	<.0001
WBDC197	119386	SYR	11.21	1.437	<.0001
WBDC198	119402	SYR	5.71	1.359	<.0001
WBDC199	119424	SYR	7.98	1.458	<.0001
WBDC200	119427	SYR	11.90	1.394	<.0001
WBDC201	119443	SYR	10.21	1.479	<.0001
WBDC202	119451	SYR	12.24	1.323	<.0001
WBDC203	119458	SYR	8.99	1.323	<.0001
WBDC204	120794	TKM	7.40	1.394	<.0001
WBDC205	120920	RUS	7.01	1.394	<.0001
WBDC206	121857	SYR	7.91	1.531	<.0001
WBDC207	123949	UZB	9.31	1.359	<.0001
WBDC208	123959	UZB	9.39	1.811	<.0001
WBDC209	123972	UZB	8.90	1.394	<.0001
WBDC210	123991	UZB	8.87	1.394	<.0001
WBDC211	124000	UZB	12.16	1.359	<.0001
WBDC212	124017	UZB	10.87	1.581	<.0001
WBDC213	124035	UZB	11.28	2.372	<.0001
WBDC214	124046	UZB	13.21	1.650	<.0001
WBDC215	126427	TKM	8.51	1.479	<.0001
WBDC216	126484	TKM	4.27	3.162	0.1769
WBDC217	126933	ARM	8.78	1.650	<.0001
WBDC218	131375	KAZ	10.64	3.240	0.001
WBDC219	131620	KAZ	11.53	1.559	<.0001

WBDC220	131642	KAZ	8.78	1.791	<.0001
WBDC221	131674	TJK	9.95	2.259	<.0001
WBDC222	131675	TJK	8.20	1.909	<.0001
WBDC223	131684	TJK	8.84	1.650	<.0001
WBDC224	131790	TJK	10.00	1.791	<.0001
WBDC225	131792	TJK	10.03	2.092	<.0001
WBDC227	132552	AZE	10.68	2.092	<.0001
WBDC228	132606	AZE	10.09	1.650	<.0001
WBDC229	132623	AZE	11.39	1.778	<.0001
WBDC230	132627	AZE	12.00	2.562	<.0001
WBDC231	132636	AZE	9.89	1.581	<.0001
WBDC232	132666	AZE	4.39	1.391	<.0001
WBDC233	38668	AFG	6.24	1.577	<.0001
WBDC234	39884	CYP	5.84	1.309	<.0001
WBDC235	40010	JOR	6.96	1.347	<.0001
WBDC236	40029	JOR	8.29	1.357	<.0001
WBDC237	40033	JOR	8.25	1.555	<.0001
WBDC238	40037	JOR	8.42	1.703	<.0001
WBDC240	135267	JOR	8.60	1.347	<.0001
WBDC241	135273	JOR	10.14	1.649	<.0001
WBDC242	38620	JOR	6.07	1.555	<.0001
WBDC243	40000	JOR	5.98	1.278	<.0001
WBDC244	135700	JOR	7.16	1.270	<.0001
WBDC245	38621	JOR	5.78	1.905	0.0033
WBDC246	38626	JOR	3.63	1.327	<.0001
WBDC247	38630	JOR	8.35	1.455	<.0001
WBDC248	39394	JOR	6.37	2.057	0.0003
WBDC250	39988	JOR	6.33	1.309	<.0001
WBDC252	40008	JOR	7.11	1.270	<.0001
WBDC253	115787	JOR	3.83	1.238	<.0001
WBDC254	115790	JOR	6.81	1.309	<.0001
WBDC255	115792	JOR	8.56	1.703	<.0001
WBDC256	135856	JOR	12.07	1.455	<.0001
WBDC257	39399	JOR	9.77	1.309	<.0001
WBDC258	40041	JOR	8.42	1.967	<.0001
WBDC259	40047	JOR	12.94	1.309	<.0001
WBDC260	39822	JOR	10.13	1.498	<.0001
WBDC261	40065	JOR	11.34	1.391	<.0001
WBDC262	40069	JOR	6.45	1.357	<.0001
WBDC263	40070	JOR	10.23	1.440	<.0001
WBDC265	40052	JOR	9.44	1.440	<.0001

WBDC266	40054	JOR	10.97	1.455	<.0001
WBDC267	40062	JOR	10.01	1.347	<.0001
WBDC268	135254	JOR	10.32	1.688	<.0001
WBDC269	39546	LBN	8.84	1.394	<.0001
WBDC270	38866	PAL	12.25	1.394	<.0001
WBDC271	38938	PAL	9.60	1.498	<.0001
WBDC274	39387	PAL	12.23	1.535	<.0001
WBDC275	39390	PAL	9.84	1.238	<.0001
WBDC276	38862	PAL	11.61	1.394	<.0001
WBDC277	38942	PAL	10.77	1.327	<.0001
WBDC278	39108	PAL	2.53	1.403	0.0712
WBDC279	39386	PAL	3.29	1.598	0.0397
WBDC280	39687	PAL	4.34	1.791	0.0155
WBDC281	38832	PAL	1.00	1.491	**0.5010***
WBDC282	38874	PAL	7.27	1.437	<.0001
WBDC283	38880	PAL	4.87	1.646	0.0031
WBDC284	38939	PAL	4.02	1.437	0.0052
WBDC285	39071	PAL	6.23	1.598	<.0001
WBDC286	39080	PAL	5.90	1.491	<.0001
WBDC287	39092	PAL	0.84	1.559	**0.5881***
WBDC288	39700	PAL	6.50	1.437	<.0001
WBDC289	39714	PAL	3.13	1.791	**0.0807***
WBDC290	38891	PAL	5.36	1.791	0.0028
WBDC291	38906	PAL	4.96	1.531	0.0012
WBDC292	38926	PAL	4.49	1.791	0.0123
WBDC293	38932	PAL	7.31	1.811	<.0001
WBDC294	38950	PAL	3.89	1.732	0.0248
WBDC295	110780	SYR	8.52	1.854	<.0001
WBDC296	135336	SYR	6.19	1.491	<.0001
WBDC297	135346	SYR	6.12	1.732	0.0004
WBDC298	135357	SYR	0.45	2.092	**0.8297***
WBDC299	39912	SYR	5.22	1.394	0.0002
WBDC300	117896	SYR	3.46	1.559	0.0265
WBDC302	38635	SYR	8.02	1.686	<.0001
WBDC303	38640	SYR	5.54	1.359	<.0001
WBDC304	40161	SYR	2.75	1.359	0.0434
WBDC305	40162	SYR	1.87	1.598	**0.2427***
WBDC306	40166	SYR	3.66	1.650	0.0264
WBDC307	40175	SYR	3.98	2.092	**0.0570***
WBDC308	40082	SYR	4.57	1.458	0.0017
WBDC309	119435	SYR	12.82	1.947	<.0001

WBDC310	39849	SYR	11.05	1.458	<.0001
WBDC311	39882	SYR	13.00	1.491	<.0001
WBDC312	40079	SYR	14.40	1.646	<.0001
WBDC314	119420	SYR	13.83	1.708	<.0001
WBDC315	119431	SYR	15.63	1.528	<.0001
WBDC316	38613	SYR	12.02	1.437	<.0001
WBDC317	39843	SYR	16.40	1.598	<.0001
WBDC318	39919	SYR	12.83	1.708	<.0001
WBDC319	40084	SYR	13.38	1.646	<.0001
WBDC320	40094	SYR	15.66	1.531	<.0001
WBDC323	135606	TKM	13.14	1.811	<.0001
WBDC324	135609	TKM	16.39	1.646	<.0001
WBDC326	38813	TKM	10.18	1.491	<.0001
WBDC329	135537	TKM	13.73	1.791	<.0001
WBDC330	135504	TKM	13.60	1.650	<.0001
WBDC331	135507	TKM	14.35	1.732	<.0001
WBDC332	135460	TKM	3.86	1.531	0.0117
WBDC333	135478	TKM	7.30	1.394	<.0001
WBDC334	135563	TKM	6.64	1.437	<.0001
WBDC335	135624	TKM	6.98	1.437	<.0001
WBDC336	126406	TKM	7.03	1.437	<.0001
WBDC337	116111	TUR	3.69	1.394	0.0082
WBDC338	116112	TUR	8.06	1.491	<.0001
WBDC340	116116	TUR	7.20	1.646	<.0001
WBDC341	116126	TUR	9.58	1.394	<.0001
WBDC342	116128	TUR	7.73	1.598	<.0001
WBDC343	116130	TUR	7.20	1.394	<.0001
WBDC344	38674	TUR	5.08	1.394	0.0003
WBDC345	40155	UZB	7.06	1.403	<.0001
WBDC346	120795	UZB	7.30	1.437	<.0001
WBDC347	40152	UZB	9.81	1.394	<.0001
WBDC348	Damon 11-11 (B)	ISR	3.15	1.531	0.04
WBDC349	Shechem 12-32 (A)	ISR	6.69	1.437	<.0001
WBDC350	41-1 (#1)	ISR	7.56	1.323	<.0001
WBDC354	HS 680		6.75	1.323	<.0001
WBDC355	OUH602		5.34	1.437	0.0002

† Estimates of differences between least square means of inoculum treatments and non-inoculated controls

§Standard error of the differences

*Differences between inoculum treatment and non-inoculated control is not significant at $P < 0.05$

Appendix 3: Comparison of estimates of differences between inoculated treatment and non-inoculated control for root weight of 317 wild barley accessions in first screening for resistance to Rhizoctonia root rot

Accession	IG #	Origin	Diff[†]	SE[§]	P value
Lenetah	Cultivated Barley		0.722	0.043	<.0001
WBDC001	38610	SYR	0.391	0.038	0.0309
WBDC002	38611	SYR	0.280	0.039	**0.1156**[*]
WBDC004	38614	SYR	0.521	0.040	0.0034
WBDC005	38616	JOR	0.370	0.044	**0.0548**[*]
WBDC006	38619	JOR	0.275	0.042	**0.1972**[*]
WBDC007	38623	JOR	0.119	0.038	**0.6851**[*]
WBDC008	38627	JOR	0.516	0.039	0.0001
WBDC009	38633	JOR	0.102	0.039	**0.9348**[*]
WBDC010	38653	AFG	0.305	0.039	0.0085
WBDC011	38655	IRQ	0.360	0.038	**0.2410**[*]
WBDC012	38656	AFG	0.516	0.041	0.0017
WBDC013	38658	IRQ	0.359	0.039	**0.0572**[*]
WBDC014	38659	AFG	0.260	0.044	**0.9196**[*]
WBDC015	38660	AFG	0.584	0.039	0.0026
WBDC016	38661	IRN	0.446	0.040	**0.0648**[*]
WBDC017	38665	SYR	0.488	0.041	0.0133
WBDC018	38670	AFG	0.365	0.038	0.0195
WBDC019	38671	IRN	0.317	0.039	**0.7497**[*]
WBDC020	38672	TUR	0.675	0.038	0.0004
WBDC021	38678	IRQ	0.309	0.039	**0.3492**[*]
WBDC022	38679	TUR	0.279	0.035	**0.1479**[*]
WBDC023	38681	IRN	0.235	0.035	0.021
WBDC024	38682	IRN	0.323	0.034	0.0017
WBDC025	38693	PAK	0.193	0.034	**0.0923**[*]
WBDC026	38826	TJK	0.291	0.035	0.0244
WBDC027	38828	AZE	0.364	0.034	0.0036
WBDC028	38840	PAL	0.535	0.034	0.0003
WBDC029	38843	PAL	0.374	0.034	0.0002
WBDC030	38853	PAL	0.278	0.034	0.0389
WBDC031	38860	PAL	0.271	0.035	0.0197
WBDC032	38869	PAL	0.331	0.035	0.0026
WBDC033	38886	PAL	0.349	0.035	0.0031
WBDC034	38912	PAL	0.247	0.034	**0.1030**[*]
WBDC035	38981	PAL	0.416	0.034	<.0001
WBDC036	39002	AFG	0.241	0.035	0.0133

WBDC037	39082	PAL	0.398	0.035	<.0001
WBDC038	39117	PAL	0.345	0.035	0.0127
WBDC039	39393	JOR	0.378	0.039	<.0001
WBDC040	39591	PAL	0.394	0.039	0.0002
WBDC041	39603	PAL	0.149	0.035	0.0665
WBDC042	39673	PAL	0.818	0.035	<.0001
WBDC043	39704	PAL	1.069	0.039	<.0001
WBDC044	39759	PAL	0.753	0.035	<.0001
WBDC045	39821	JOR	0.748	0.034	<.0001
WBDC046	39824	JOR	0.691	0.035	<.0001
WBDC047	39825	JOR	0.818	0.043	<.0001
WBDC048	39837	TUR	0.808	0.034	<.0001
WBDC049	39839	TUR	0.782	0.035	<.0001
WBDC050	39846	SYR	0.728	0.038	<.0001
WBDC051	39847	SYR	0.828	0.041	<.0001
WBDC052	39850	JOR	0.819	0.035	<.0001
WBDC053	39852	PAK	0.903	0.035	<.0001
WBDC054	39857	SYR	0.660	0.035	<.0001
WBDC055	39859	SYR	0.683	0.034	<.0001
WBDC056	39876	TUR	0.930	0.039	<.0001
WBDC057	39880	SYR	0.491	0.042	<.0001
WBDC058	39885	CYP	0.618	0.037	<.0001
WBDC059	39886	CYP	0.597	0.035	<.0001
WBDC060	39891	EGY	0.738	0.035	<.0001
WBDC061	39910	SYR	0.814	0.035	<.0001
WBDC062	39911	SYR	0.197	0.034	0.0442
WBDC063	39913	SYR	0.139	0.034	0.0437
WBDC064	39914	SYR	0.305	0.036	**0.2724***
WBDC065	39915	SYR	0.003	0.034	<.0001
WBDC066	39916	SYR	0.129	0.036	0.033
WBDC067	39917	SYR	0.184	0.035	**0.5282***
WBDC068	39918	SYR	0.136	0.037	**0.3881***
WBDC069	39920	SYR	-0.006	0.035	0.0003
WBDC070	39932	SYR	0.077	0.037	0.0003
WBDC072	39935	LBY	0.077	0.035	<.0001
WBDC073	39936	LBY	0.043	0.034	<.0001
WBDC074	39937	LBY	-0.010	0.035	<.0001
WBDC075	39939	LBY	0.319	0.034	**0.3500***
WBDC078	39942	SYR	0.236	0.040	**0.6525***
WBDC079	39990	JOR	0.029	0.036	0.0012
WBDC080	39996	JOR	0.099	0.034	0.0234

WBDC081	40002	JOR	0.300	0.034	**0.3647***
WBDC082	40009	JOR	0.222	0.034	**0.1666***
WBDC083	40012	JOR	0.101	0.034	0.0026
WBDC085	40015	JOR	0.083	0.034	0.0402
WBDC089	40026	JOR	0.303	0.045	0.0406
WBDC092	40034	JOR	0.445	0.041	**0.2709***
WBDC093	40035	JOR	0.356	0.045	**0.1481***
WBDC094	40039	JOR	0.201	0.043	**0.8438***
WBDC095	40045	JOR	0.008	0.041	0.0056
WBDC097	40051	JOR	0.434	0.046	**0.1588***
WBDC100	40060	JOR	0.189	0.042	**0.9484***
WBDC101	40063	JOR	0.293	0.042	**0.3509***
WBDC102	40064	JOR	0.095	0.043	**0.3238***
WBDC103	40071	JOR	0.100	0.041	0.0007
WBDC104	40072	JOR	0.339	0.043	**0.2276***
WBDC105	40075	JOR	0.236	0.051	**0.8296***
WBDC106	40077	SYR	0.089	0.038	0.0064
WBDC107	40078	SYR	0.177	0.048	**0.8642***
WBDC108	40080	SYR	0.177	0.044	0.0482
WBDC109	40090	SYR	0.365	0.045	**0.2819***
WBDC110	40091	SYR	0.412	0.044	0.0046
WBDC111	40097	SYR	0.108	0.042	**0.4465***
WBDC112	40098	SYR	0.266	0.043	**0.6527***
WBDC113	40099	TKM	0.161	0.043	**0.2079***
WBDC115	40104	TKM	0.211	0.042	**0.6407***
WBDC116	40105	TKM	0.418	0.043	<.0001
WBDC117	40106	TKM	0.646	0.042	<.0001
WBDC119	40108	UZB	0.113	0.046	**0.1591***
WBDC120	40109	TJK	0.302	0.038	0.0663
WBDC121	40138	IRN	0.150	0.041	**0.5178***
WBDC122	40140	IRN	0.322	0.039	0.0135
WBDC123	40142	IRN	0.190	0.045	**0.3549***
WBDC124	40143	IRN	0.342	0.044	0.0101
WBDC125	40154	UZB	0.410	0.045	0.0072
WBDC126	40156	LBN	0.636	0.035	<.0001
WBDC127	40159	SYR	0.588	0.038	<.0001
WBDC128	40164	SYR	0.641	0.037	<.0001
WBDC129	40171	SYR	1.028	0.053	<.0001
WBDC130	40173	SYR	0.588	0.036	<.0001
WBDC131	40174	SYR	0.799	0.039	<.0001
WBDC132	40177	LBN	0.667	0.038	<.0001

WBDC133	40178	LBN	0.599	0.036	<.0001
WBDC134	40179	LBN	0.542	0.042	<.0001
WBDC135	40180	LBN	0.582	0.036	<.0001
WBDC136	40181	LBN	0.748	0.035	<.0001
WBDC137	40182	LBN	0.703	0.040	<.0001
WBDC138	40183	LBN	0.258	0.036	0.0007
WBDC139	40184	LBN	0.633	0.035	<.0001
WBDC140	40186	LBN	0.610	0.036	<.0001
WBDC141	40187	LBN	0.752	0.035	<.0001
WBDC142	40188	LBN	0.528	0.037	<.0001
WBDC143	40191	LBN	0.463	0.035	<.0001
WBDC145	40194	LBN	0.562	0.036	<.0001
WBDC146	40196	IRN	0.370	0.039	<.0001
WBDC147	40197	IRN	0.440	0.038	<.0001
WBDC148	40198	IRN	0.396	0.043	<.0001
WBDC149	40199	IRN	0.335	0.043	0.0005
WBDC150	40200	IRN	0.724	0.039	<.0001
WBDC151	40201	SYR	0.918	0.037	<.0001
WBDC152	107046	IRN	0.661	0.039	<.0001
WBDC153	107047	IRN	0.811	0.038	<.0001
WBDC154	107423	IRQ	0.540	0.043	<.0001
WBDC155	107424	IRQ	0.635	0.041	<.0001
WBDC156	107425	IRQ	0.686	0.036	<.0001
WBDC157	107426	IRQ	1.032	0.044	<.0001
WBDC158	107427	IRQ	0.483	0.040	<.0001
WBDC159	110739	SYR	0.916	0.043	<.0001
WBDC160	110742	SYR	0.759	0.044	<.0001
WBDC161	110751	SYR	0.294	0.037	<.0001
WBDC164	110773	SYR	0.709	0.043	<.0001
WBDC165	110793	SYR	0.881	0.037	<.0001
WBDC166	110798	SYR	0.913	0.040	<.0001
WBDC167	110804	SYR	0.939	0.041	<.0001
WBDC168	110816	LBN	0.868	0.039	<.0001
WBDC169	110819	LBN	0.540	0.039	<.0001
WBDC170	110831	LBN	0.934	0.037	<.0001
WBDC171	110833	LBN	0.815	0.039	<.0001
WBDC172	112673	IRN	0.894	0.042	<.0001
WBDC173	112674	IRN	0.930	0.035	<.0001
WBDC174	112679	IRN	0.605	0.039	<.0001
WBDC177	112797	IRQ	0.835	0.037	<.0001
WBDC178	112813	IRQ	0.619	0.043	<.0001

WBDC179	112846	LBY	0.627	0.037	<.0001
WBDC180	112847	LBY	0.443	0.038	<.0001
WBDC181	115780	JOR	0.436	0.041	<.0001
WBDC182	115781	JOR	0.476	0.038	<.0001
WBDC183	115789	JOR	0.450	0.039	<.0001
WBDC184	116004	LBY	0.483	0.039	<.0001
WBDC185	116005	LBY	0.293	0.043	0.0476
WBDC186	116099	TUR	0.661	0.042	<.0001
WBDC187	116100	TUR	0.321	0.046	<.0001
WBDC188	116104	TUR	0.544	0.039	<.0001
WBDC189	116105	TUR	0.518	0.042	<.0001
WBDC190	116106	TUR	0.583	0.039	<.0001
WBDC191	116107	TUR	0.535	0.040	<.0001
WBDC192	116108	TUR	0.418	0.041	<.0001
WBDC193	116118	TUR	0.555	0.038	<.0001
WBDC194	116119	TUR	0.516	0.046	<.0001
WBDC195	116121	TUR	0.187	0.058	**0.0702***
WBDC196	116125	TUR	0.463	0.039	<.0001
WBDC197	119386	SYR	0.447	0.042	<.0001
WBDC198	119402	SYR	0.472	0.039	<.0001
WBDC199	119424	SYR	0.437	0.042	<.0001
WBDC200	119427	SYR	0.671	0.040	<.0001
WBDC201	119443	SYR	0.613	0.043	<.0001
WBDC202	119451	SYR	0.590	0.038	<.0001
WBDC203	119458	SYR	0.509	0.038	<.0001
WBDC204	120794	TKM	0.671	0.040	<.0001
WBDC205	120920	RUS	0.345	0.040	0.0004
WBDC206	121857	SYR	0.766	0.044	<.0001
WBDC207	123949	UZB	0.448	0.039	<.0001
WBDC208	123959	UZB	0.482	0.053	<.0001
WBDC209	123972	UZB	0.556	0.040	<.0001
WBDC210	123991	UZB	0.508	0.040	<.0001
WBDC211	124000	UZB	0.743	0.039	<.0001
WBDC212	124017	UZB	0.648	0.046	<.0001
WBDC213	124035	UZB	0.572	0.069	<.0001
WBDC214	124046	UZB	0.677	0.048	<.0001
WBDC215	126427	TKM	0.360	0.043	0.013
WBDC216	126484	TKM	0.289	0.092	**0.2991***
WBDC217	126933	ARM	0.407	0.048	0.018
WBDC218	131375	KAZ	0.428	0.094	0.0617
WBDC219	131620	KAZ	0.572	0.045	<.0001

WBDC220	131642	KAZ	0.583	0.052	<.0001
WBDC221	131674	TJK	0.455	0.065	**0.1493***
WBDC222	131675	TJK	0.489	0.055	0.0014
WBDC223	131684	TJK	0.481	0.048	0.0065
WBDC224	131790	TJK	0.408	0.052	**0.0611***
WBDC225	131792	TJK	0.385	0.061	**0.6977***
WBDC227	132552	AZE	0.507	0.061	0.013
WBDC228	132606	AZE	0.552	0.048	<.0001
WBDC229	132623	AZE	0.620	0.052	0.0002
WBDC230	132627	AZE	0.444	0.074	0.0075
WBDC231	132636	AZE	0.442	0.046	0.0113
WBDC232	132666	AZE	0.897	0.039	<.0001
WBDC233	38668	AFG	0.560	0.044	<.0001
WBDC234	39884	CYP	0.884	0.037	<.0001
WBDC235	40010	JOR	0.857	0.038	<.0001
WBDC236	40029	JOR	0.729	0.038	<.0001
WBDC237	40033	JOR	0.880	0.043	<.0001
WBDC238	40037	JOR	0.792	0.048	<.0001
WBDC240	135267	JOR	0.635	0.038	<.0001
WBDC241	135273	JOR	0.856	0.046	<.0001
WBDC242	38620	JOR	0.806	0.043	<.0001
WBDC243	40000	JOR	0.762	0.036	<.0001
WBDC244	135700	JOR	1.081	0.035	<.0001
WBDC245	38621	JOR	0.311	0.053	<.0001
WBDC246	38626	JOR	0.476	0.037	<.0001
WBDC247	38630	JOR	0.761	0.041	<.0001
WBDC248	39394	JOR	0.548	0.057	0.0002
WBDC250	39988	JOR	0.582	0.037	<.0001
WBDC252	40008	JOR	0.870	0.035	<.0001
WBDC253	115787	JOR	0.532	0.035	<.0001
WBDC254	115790	JOR	0.861	0.037	<.0001
WBDC255	115792	JOR	0.672	0.048	<.0001
WBDC256	135856	JOR	0.630	0.041	<.0001
WBDC257	39399	JOR	0.758	0.037	<.0001
WBDC258	40041	JOR	0.536	0.055	0.0001
WBDC259	40047	JOR	0.691	0.037	<.0001
WBDC260	39822	JOR	0.459	0.042	<.0001
WBDC261	40065	JOR	0.747	0.039	<.0001
WBDC262	40069	JOR	0.574	0.038	<.0001
WBDC263	40070	JOR	0.775	0.040	<.0001
WBDC265	40052	JOR	0.560	0.040	<.0001

WBDC266	40054	JOR	0.509	0.041	0.0012
WBDC267	40062	JOR	0.684	0.038	<.0001
WBDC268	135254	JOR	0.544	0.047	<.0001
WBDC269	39546	LBN	0.479	0.039	<.0001
WBDC270	38866	PAL	0.753	0.039	<.0001
WBDC271	38938	PAL	0.499	0.042	<.0001
WBDC274	39387	PAL	0.880	0.043	<.0001
WBDC275	39390	PAL	0.879	0.035	<.0001
WBDC276	38862	PAL	0.813	0.039	<.0001
WBDC277	38942	PAL	0.591	0.037	<.0001
WBDC278	39108	PAL	0.217	0.041	0.0135
WBDC279	39386	PAL	0.385	0.046	0.0003
WBDC280	39687	PAL	0.120	0.052	**0.7376***
WBDC281	38832	PAL	0.178	0.043	**0.1721***
WBDC282	38874	PAL	0.439	0.042	0.003
WBDC283	38880	PAL	0.266	0.048	0.0491
WBDC284	38939	PAL	0.214	0.042	0.0096
WBDC285	39071	PAL	0.389	0.046	0.0026
WBDC286	39080	PAL	0.412	0.043	<.0001
WBDC287	39092	PAL	-0.006	0.045	0.1326
WBDC288	39700	PAL	0.421	0.042	0.0002
WBDC289	39714	PAL	0.133	0.052	**0.8450***
WBDC290	38891	PAL	0.456	0.052	<.0001
WBDC291	38906	PAL	0.280	0.044	**0.3960***
WBDC292	38926	PAL	0.381	0.052	0.003
WBDC293	38932	PAL	0.412	0.053	0.0013
WBDC294	38950	PAL	0.280	0.050	**0.1898***
WBDC295	110780	SYR	0.362	0.054	0.001
WBDC296	135336	SYR	0.235	0.043	**0.1450***
WBDC297	135346	SYR	0.275	0.050	0.0459
WBDC298	135357	SYR	0.052	0.061	**0.1460***
WBDC299	39912	SYR	0.237	0.040	**0.1027***
WBDC300	117896	SYR	0.359	0.045	0.0011
WBDC302	38635	SYR	0.423	0.049	0.0002
WBDC303	38640	SYR	0.310	0.039	0.0061
WBDC304	40161	SYR	0.281	0.039	0.0156
WBDC305	40162	SYR	0.039	0.046	**0.5556***
WBDC306	40166	SYR	0.296	0.048	0.0076
WBDC307	40175	SYR	0.280	0.061	**0.0896***
WBDC308	40082	SYR	0.321	0.042	0.0006
WBDC309	119435	SYR	0.230	0.056	0.0428

WBDC310	39849	SYR	0.296	0.042	**0.0887***
WBDC311	39882	SYR	0.286	0.043	0.0056
WBDC312	40079	SYR	0.278	0.048	0.0067
WBDC314	119420	SYR	0.379	0.050	0.0006
WBDC315	119431	SYR	0.516	0.044	<.0001
WBDC316	38613	SYR	0.377	0.042	0.0013
WBDC317	39843	SYR	0.455	0.046	0.0021
WBDC318	39919	SYR	0.258	0.050	**0.2164***
WBDC319	40084	SYR	0.485	0.048	<.0001
WBDC320	40094	SYR	0.445	0.044	0.0014
WBDC323	135606	TKM	0.364	0.053	0.003
WBDC324	135609	TKM	0.523	0.048	<.0001
WBDC326	38813	TKM	0.259	0.043	**0.0720***
WBDC329	135537	TKM	0.424	0.052	0.0002
WBDC330	135504	TKM	0.326	0.048	0.0132
WBDC331	135507	TKM	0.460	0.050	<.0001
WBDC332	135460	TKM	0.283	0.044	0.0001
WBDC333	135478	TKM	0.358	0.040	0.0004
WBDC334	135563	TKM	0.358	0.042	0.0004
WBDC335	135624	TKM	0.214	0.042	0.0002
WBDC336	126406	TKM	0.323	0.042	0.0002
WBDC337	116111	TUR	0.723	0.040	<.0001
WBDC338	116112	TUR	0.372	0.043	<.0001
WBDC340	116116	TUR	0.462	0.048	<.0001
WBDC341	116126	TUR	0.298	0.040	**0.0781***
WBDC342	116128	TUR	0.243	0.046	0.0197
WBDC343	116130	TUR	0.412	0.040	<.0001
WBDC344	38674	TUR	0.474	0.040	<.0001
WBDC345	40155	UZB	0.430	0.041	0.0002
WBDC346	120795	UZB	0.417	0.042	0.0002
WBDC347	40152	UZB	0.557	0.040	<.0001
WBDC348	Damon 11-11 (B)	ISR	0.224	0.044	<.0001
WBDC349	Shechem 12-32 (A)	ISR	0.479	0.042	<.0001
WBDC350	41-1 (#1)	ISR	0.495	0.038	<.0001
WBDC354	HS 680		0.680	0.038	<.0001
WBDC355	OUH602		0.078	0.042	**0.0615***

† Estimates of differences between least square means of inoculum treatments and non-inoculated controls

§Standard error of the differences

*Differences between inoculum treatment and non-inoculated control is not significant at $P < 0.05$

Appendix 4: Comparison of estimates of differences between inoculated treatment and non-inoculated control for shoot length of 317 accessions in first screening for resistance to Rhizoctonia root rot

Accession	IG #	Origin	Diff[†]	SE[§]	P value
Lenetah	Cultivated Barley	USA	0.274	0.049	<.0001
WBDC001	38610	SYR	0.098	0.043	<.0001
WBDC002	38611	SYR	0.074	0.045	0.0026
WBDC004	38614	SYR	0.156	0.046	<.0001
WBDC005	38616	JOR	0.117	0.050	0.003
WBDC006	38619	JOR	0.065	0.047	0.0062
WBDC007	38623	JOR	-0.016	0.043	**0.1420**[*]
WBDC008	38627	JOR	0.185	0.045	<.0001
WBDC009	38633	JOR	-0.004	0.045	**0.3068**[*]
WBDC010	38653	AFG	0.116	0.045	0.0003
WBDC011	38655	IRQ	0.055	0.043	0.0002
WBDC012	38656	AFG	0.149	0.046	<.0001
WBDC013	38658	IRQ	0.100	0.045	0.0004
WBDC014	38659	AFG	-0.005	0.050	0.0107
WBDC015	38660	AFG	0.152	0.045	<.0001
WBDC016	38661	IRN	0.102	0.046	<.0001
WBDC017	38665	SYR	0.130	0.046	<.0001
WBDC018	38670	AFG	0.092	0.043	<.0001
WBDC019	38671	IRN	0.014	0.045	0.0004
WBDC020	38672	TUR	0.210	0.043	<.0001
WBDC021	38678	IRQ	0.056	0.045	0.0068
WBDC022	38679	TUR	0.049	0.045	<.0001
WBDC023	38681	IRN	0.077	0.045	0.0082
WBDC024	38682	IRN	0.089	0.043	0.0022
WBDC025	38693	PAK	0.039	0.043	<.0001
WBDC026	38826	TJK	0.089	0.045	**0.1102**[*]
WBDC027	38828	AZE	0.100	0.043	0.0006
WBDC028	38840	PAL	0.116	0.043	<.0001
WBDC029	38843	PAL	0.124	0.043	0.0002
WBDC030	38853	PAL	0.054	0.043	<.0001
WBDC031	38860	PAL	0.074	0.045	0.001
WBDC032	38869	PAL	0.083	0.045	<.0001
WBDC033	38886	PAL	0.116	0.045	0.0012
WBDC034	38912	PAL	0.055	0.043	0.0005
WBDC035	38981	PAL	0.133	0.043	<.0001
WBDC036	39002	AFG	0.075	0.045	0.0007

WBDC037	39082	PAL	0.134	0.045	<.0001
WBDC038	39117	PAL	0.079	0.045	<.0001
WBDC039	39393	JOR	0.144	0.050	0.0183
WBDC040	39591	PAL	0.122	0.050	<.0001
WBDC041	39603	PAL	0.056	0.045	0.003
WBDC042	39673	PAL	0.464	0.045	<.0001
WBDC043	39704	PAL	0.560	0.050	<.0001
WBDC044	39759	PAL	0.449	0.045	**0.0587***
WBDC045	39821	JOR	0.362	0.043	<.0001
WBDC046	39824	JOR	0.401	0.045	**0.1394***
WBDC047	39825	JOR	0.365	0.055	<.0001
WBDC048	39837	TUR	0.472	0.043	0.0017
WBDC049	39839	TUR	0.447	0.045	<.0001
WBDC050	39846	SYR	0.403	0.049	0.0004
WBDC051	39847	SYR	0.470	0.052	<.0001
WBDC052	39850	JOR	0.535	0.045	0.0011
WBDC053	39852	PAK	0.530	0.045	<.0001
WBDC054	39857	SYR	0.381	0.045	<.0001
WBDC055	39859	SYR	0.348	0.043	<.0001
WBDC056	39876	TUR	0.532	0.050	<.0001
WBDC057	39880	SYR	0.226	0.054	<.0001
WBDC058	39885	CYP	0.275	0.047	<.0001
WBDC059	39886	CYP	0.382	0.046	**0.0689***
WBDC060	39891	EGY	0.408	0.045	<.0001
WBDC061	39910	SYR	0.477	0.046	0.0003
WBDC062	39911	SYR	-0.049	0.043	<.0001
WBDC063	39913	SYR	-0.054	0.043	**0.1101***
WBDC064	39914	SYR	0.033	0.046	<.0001
WBDC065	39915	SYR	-0.116	0.043	**0.2604***
WBDC066	39916	SYR	-0.064	0.046	<.0001
WBDC067	39917	SYR	-0.017	0.045	0.0003
WBDC068	39918	SYR	-0.025	0.047	<.0001
WBDC069	39920	SYR	-0.098	0.045	**0.2139***
WBDC070	39932	SYR	-0.083	0.047	0.0006
WBDC072	39935	LBY	-0.095	0.045	0.0478
WBDC073	39936	LBY	-0.086	0.043	**0.1215***
WBDC074	39937	LBY	-0.118	0.045	0.0002
WBDC075	39939	LBY	0.029	0.043	0.0078
WBDC078	39942	SYR	0.019	0.052	0.0455
WBDC079	39990	JOR	-0.104	0.046	0.0115
WBDC080	39996	JOR	-0.081	0.043	0.0164

WBDC081	40002	JOR	0.034	0.043	<.0001
WBDC082	40009	JOR	-0.039	0.043	<.0001
WBDC083	40012	JOR	-0.074	0.043	0.0036
WBDC085	40015	JOR	-0.072	0.043	**0.1025***
WBDC089	40026	JOR	0.060	0.051	<.0001
WBDC092	40034	JOR	0.046	0.046	<.0001
WBDC093	40035	JOR	0.043	0.051	<.0001
WBDC094	40039	JOR	0.006	0.049	0.0055
WBDC095	40045	JOR	-0.092	0.046	**0.9128***
WBDC097	40051	JOR	0.051	0.052	<.0001
WBDC100	40060	JOR	0.002	0.047	0.0032
WBDC101	40063	JOR	0.026	0.047	<.0001
WBDC102	40064	JOR	-0.020	0.049	**0.0902***
WBDC103	40071	JOR	-0.108	0.046	**0.1314***
WBDC104	40072	JOR	0.043	0.049	<.0001
WBDC105	40075	JOR	0.006	0.057	0.0021
WBDC106	40077	SYR	-0.058	0.043	**0.1249***
WBDC107	40078	SYR	0.005	0.054	0.0192
WBDC108	40080	SYR	0.028	0.050	<.0001
WBDC109	40090	SYR	0.047	0.051	0.0003
WBDC110	40091	SYR	0.102	0.050	<.0001
WBDC111	40097	SYR	-0.023	0.048	**0.1397***
WBDC112	40098	SYR	0.014	0.048	0.0004
WBDC113	40099	TKM	0.039	0.048	0.0246
WBDC115	40104	TKM	0.016	0.047	0.0058
WBDC116	40105	TKM	0.150	0.049	<.0001
WBDC117	40106	TKM	0.209	0.047	<.0001
WBDC119	40108	UZB	-0.037	0.052	**0.1174***
WBDC120	40109	TJK	0.064	0.043	<.0001
WBDC121	40138	IRN	-0.016	0.046	0.0159
WBDC122	40140	IRN	0.056	0.045	<.0001
WBDC123	40142	IRN	0.028	0.051	0.01
WBDC124	40143	IRN	0.086	0.050	<.0001
WBDC125	40154	UZB	0.085	0.051	<.0001
WBDC126	40156	LBN	0.375	0.045	<.0001
WBDC127	40159	SYR	0.309	0.049	<.0001
WBDC128	40164	SYR	0.338	0.047	<.0001
WBDC129	40171	SYR	0.638	0.069	<.0001
WBDC130	40173	SYR	0.337	0.046	<.0001
WBDC131	40174	SYR	0.464	0.050	<.0001
WBDC132	40177	LBN	0.396	0.049	<.0001

WBDC133	40178	LBN	0.323	0.046	<.0001
WBDC134	40179	LBN	0.326	0.054	0.0006
WBDC135	40180	LBN	0.355	0.046	<.0001
WBDC136	40181	LBN	0.495	0.045	<.0001
WBDC137	40182	LBN	0.404	0.052	<.0001
WBDC138	40183	LBN	0.122	0.046	0.001
WBDC139	40184	LBN	0.381	0.045	<.0001
WBDC140	40186	LBN	0.405	0.046	0.0002
WBDC141	40187	LBN	0.467	0.045	<.0001
WBDC142	40188	LBN	0.331	0.047	<.0001
WBDC143	40191	LBN	0.303	0.045	<.0001
WBDC145	40194	LBN	0.348	0.046	<.0001
WBDC146	40196	IRN	0.187	0.050	<.0001
WBDC147	40197	IRN	0.253	0.049	**0.1103***
WBDC148	40198	IRN	0.248	0.056	0.0001
WBDC149	40199	IRN	0.168	0.055	0.0033
WBDC150	40200	IRN	0.475	0.050	0.0005
WBDC151	40201	SYR	0.586	0.047	<.0001
WBDC152	107046	IRN	0.353	0.050	<.0001
WBDC153	107047	IRN	0.480	0.049	<.0001
WBDC154	107423	IRQ	0.284	0.056	<.0001
WBDC155	107424	IRQ	0.385	0.052	<.0001
WBDC156	107425	IRQ	0.407	0.046	<.0001
WBDC157	107426	IRQ	0.586	0.057	<.0001
WBDC158	107427	IRQ	0.240	0.051	<.0001
WBDC159	110739	SYR	0.515	0.056	<.0001
WBDC160	110742	SYR	0.447	0.057	<.0001
WBDC161	110751	SYR	0.278	0.047	0.0017
WBDC164	110773	SYR	0.356	0.055	<.0001
WBDC165	110793	SYR	0.588	0.047	<.0001
WBDC166	110798	SYR	0.562	0.051	<.0001
WBDC167	110804	SYR	0.587	0.052	<.0001
WBDC168	110816	LBN	0.495	0.050	0.0006
WBDC169	110819	LBN	0.384	0.050	0.0007
WBDC170	110831	LBN	0.707	0.047	<.0001
WBDC171	110833	LBN	0.461	0.050	<.0001
WBDC172	112673	IRN	0.564	0.054	<.0001
WBDC173	112674	IRN	0.616	0.046	<.0001
WBDC174	112679	IRN	0.382	0.050	0.0007
WBDC177	112797	IRQ	0.519	0.048	<.0001
WBDC178	112813	IRQ	0.291	0.056	<.0001

WBDC179	112846	LBY	0.393	0.048	0.0027
WBDC180	112847	LBY	0.288	0.049	0.0191
WBDC181	115780	JOR	0.174	0.046	<.0001
WBDC182	115781	JOR	0.192	0.043	<.0001
WBDC183	115789	JOR	0.248	0.045	<.0001
WBDC184	116004	LBY	0.243	0.045	<.0001
WBDC185	116005	LBY	0.072	0.048	0.0006
WBDC186	116099	TUR	0.319	0.048	<.0001
WBDC187	116100	TUR	0.145	0.052	<.0001
WBDC188	116104	TUR	0.184	0.045	<.0001
WBDC189	116105	TUR	0.227	0.047	<.0001
WBDC190	116106	TUR	0.271	0.045	<.0001
WBDC191	116107	TUR	0.213	0.046	<.0001
WBDC192	116108	TUR	0.141	0.046	<.0001
WBDC193	116118	TUR	0.292	0.043	<.0001
WBDC194	116119	TUR	0.270	0.052	<.0001
WBDC195	116121	TUR	0.069	0.065	**0.0805***
WBDC196	116125	TUR	0.222	0.045	<.0001
WBDC197	119386	SYR	0.200	0.047	<.0001
WBDC198	119402	SYR	0.263	0.045	<.0001
WBDC199	119424	SYR	0.173	0.048	<.0001
WBDC200	119427	SYR	0.323	0.046	<.0001
WBDC201	119443	SYR	0.225	0.048	<.0001
WBDC202	119451	SYR	0.258	0.043	<.0001
WBDC203	119458	SYR	0.321	0.043	<.0001
WBDC204	120794	TKM	0.377	0.046	<.0001
WBDC205	120920	RUS	0.152	0.046	0.0002
WBDC206	121857	SYR	0.361	0.050	<.0001
WBDC207	123949	UZB	0.166	0.045	<.0001
WBDC208	123959	UZB	0.202	0.059	<.0001
WBDC209	123972	UZB	0.225	0.046	<.0001
WBDC210	123991	UZB	0.220	0.046	<.0001
WBDC211	124000	UZB	0.247	0.045	<.0001
WBDC212	124017	UZB	0.265	0.052	<.0001
WBDC213	124035	UZB	0.232	0.078	<.0001
WBDC214	124046	UZB	0.241	0.054	<.0001
WBDC215	126427	TKM	0.089	0.048	<.0001
WBDC216	126484	TKM	0.077	0.104	**0.1141***
WBDC217	126933	ARM	0.095	0.054	<.0001
WBDC218	131375	KAZ	0.128	0.106	0.0057
WBDC219	131620	KAZ	0.249	0.051	<.0001

WBDC220	131642	KAZ	0.236	0.059	<.0001
WBDC221	131674	TJK	0.081	0.074	0.0007
WBDC222	131675	TJK	0.142	0.063	<.0001
WBDC223	131684	TJK	0.120	0.054	<.0001
WBDC224	131790	TJK	0.082	0.059	<.0001
WBDC225	131792	TJK	-0.018	0.069	0.0001
WBDC227	132552	AZE	0.130	0.069	<.0001
WBDC228	132606	AZE	0.201	0.054	<.0001
WBDC229	132623	AZE	0.189	0.058	<.0001
WBDC230	132627	AZE	0.170	0.084	0.0033
WBDC231	132636	AZE	0.110	0.052	<.0001
WBDC232	132666	AZE	0.574	0.050	0.0014
WBDC233	38668	AFG	0.370	0.057	<.0001
WBDC234	39884	CYP	0.571	0.047	<.0001
WBDC235	40010	JOR	0.542	0.049	<.0001
WBDC236	40029	JOR	0.414	0.049	<.0001
WBDC237	40033	JOR	0.486	0.056	<.0001
WBDC238	40037	JOR	0.427	0.061	<.0001
WBDC240	135267	JOR	0.349	0.049	<.0001
WBDC241	135273	JOR	0.492	0.060	<.0001
WBDC242	38620	JOR	0.477	0.056	<.0001
WBDC243	40000	JOR	0.431	0.046	<.0001
WBDC244	135700	JOR	0.624	0.046	<.0001
WBDC245	38621	JOR	0.346	0.069	0.001
WBDC246	38626	JOR	0.266	0.048	0.0056
WBDC247	38630	JOR	0.425	0.052	<.0001
WBDC248	39394	JOR	0.292	0.074	0.0019
WBDC250	39988	JOR	0.290	0.047	<.0001
WBDC252	40008	JOR	0.543	0.046	<.0001
WBDC253	115787	JOR	0.399	0.045	0.0013
WBDC254	115790	JOR	0.521	0.047	<.0001
WBDC255	115792	JOR	0.267	0.061	<.0001
WBDC256	135856	JOR	0.236	0.052	<.0001
WBDC257	39399	JOR	0.309	0.047	<.0001
WBDC258	40041	JOR	0.183	0.071	<.0001
WBDC259	40047	JOR	0.255	0.047	<.0001
WBDC260	39822	JOR	0.136	0.054	<.0001
WBDC261	40065	JOR	0.171	0.050	<.0001
WBDC262	40069	JOR	0.287	0.049	<.0001
WBDC263	40070	JOR	0.292	0.052	<.0001
WBDC265	40052	JOR	0.231	0.052	<.0001

WBDC266	40054	JOR	0.128	0.052	<.0001
WBDC267	40062	JOR	0.213	0.049	<.0001
WBDC268	135254	JOR	0.155	0.061	<.0001
WBDC269	39546	LBN	0.126	0.050	<.0001
WBDC270	38866	PAL	0.267	0.050	<.0001
WBDC271	38938	PAL	0.161	0.054	<.0001
WBDC274	39387	PAL	0.391	0.055	<.0001
WBDC275	39390	PAL	0.343	0.045	<.0001
WBDC276	38862	PAL	0.219	0.050	<.0001
WBDC277	38942	PAL	0.233	0.048	<.0001
WBDC278	39108	PAL	0.053	0.046	<.0001
WBDC279	39386	PAL	0.117	0.052	<.0001
WBDC280	39687	PAL	-0.008	0.059	**0.0728***
WBDC281	38832	PAL	0.037	0.049	0.0065
WBDC282	38874	PAL	0.102	0.047	<.0001
WBDC283	38880	PAL	0.048	0.054	<.0001
WBDC284	38939	PAL	0.054	0.047	0.0003
WBDC285	39071	PAL	0.077	0.052	<.0001
WBDC286	39080	PAL	0.123	0.049	<.0001
WBDC287	39092	PAL	-0.028	0.051	**0.8962***
WBDC288	39700	PAL	0.124	0.047	<.0001
WBDC289	39714	PAL	0.005	0.059	0.0502*
WBDC290	38891	PAL	0.142	0.059	<.0001
WBDC291	38906	PAL	0.024	0.050	0.0004
WBDC292	38926	PAL	0.079	0.059	<.0001
WBDC293	38932	PAL	0.089	0.059	<.0001
WBDC294	38950	PAL	0.035	0.057	0.0001
WBDC295	110780	SYR	0.124	0.061	<.0001
WBDC296	135336	SYR	0.036	0.049	0.0001
WBDC297	135346	SYR	0.058	0.057	0.0003
WBDC298	135357	SYR	0.044	0.069	**0.4790***
WBDC299	39912	SYR	0.041	0.046	0.0001
WBDC300	117896	SYR	0.127	0.051	<.0001
WBDC302	38635	SYR	0.105	0.055	<.0001
WBDC303	38640	SYR	0.081	0.045	<.0001
WBDC304	40161	SYR	0.060	0.045	<.0001
WBDC305	40162	SYR	-0.018	0.052	**0.5883***
WBDC306	40166	SYR	0.066	0.054	<.0001
WBDC307	40175	SYR	0.060	0.069	0.0034
WBDC308	40082	SYR	0.073	0.048	<.0001
WBDC309	119435	SYR	0.045	0.064	0.0001

WBDC310	39849	SYR	0.035	0.048	<.0001
WBDC311	39882	SYR	0.071	0.049	<.0001
WBDC312	40079	SYR	0.056	0.054	<.0001
WBDC314	119420	SYR	0.079	0.056	<.0001
WBDC315	119431	SYR	0.146	0.050	<.0001
WBDC316	38613	SYR	0.078	0.047	<.0001
WBDC317	39843	SYR	0.086	0.052	<.0001
WBDC318	39919	SYR	0.028	0.056	<.0001
WBDC319	40084	SYR	0.125	0.054	<.0001
WBDC320	40094	SYR	0.075	0.050	<.0001
WBDC323	135606	TKM	0.097	0.059	<.0001
WBDC324	135609	TKM	0.128	0.054	<.0001
WBDC326	38813	TKM	0.040	0.049	<.0001
WBDC329	135537	TKM	0.105	0.059	<.0001
WBDC330	135504	TKM	0.054	0.054	<.0001
WBDC331	135507	TKM	0.137	0.057	<.0001
WBDC332	135460	TKM	0.158	0.050	0.0011
WBDC333	135478	TKM	0.139	0.046	<.0001
WBDC334	135563	TKM	0.143	0.047	<.0001
WBDC335	135624	TKM	0.122	0.047	0.0019
WBDC336	126406	TKM	0.134	0.047	<.0001
WBDC337	116111	TUR	0.460	0.046	<.0001
WBDC338	116112	TUR	0.139	0.049	<.0001
WBDC340	116116	TUR	0.225	0.054	<.0001
WBDC341	116126	TUR	0.065	0.046	0.0001
WBDC342	116128	TUR	0.082	0.052	0.0017
WBDC343	116130	TUR	0.218	0.046	<.0001
WBDC344	38674	TUR	0.212	0.046	<.0001
WBDC345	40155	UZB	0.164	0.046	<.0001
WBDC346	120795	UZB	0.171	0.047	<.0001
WBDC347	40152	UZB	0.240	0.046	<.0001
WBDC348	Damon 11-11 (B)	ISR	0.222	0.050	0.0121
WBDC349	Shechem 12-32 (A)	ISR	0.221	0.047	<.0001
WBDC350	41-1 (#1)	ISR	0.171	0.043	<.0001
WBDC354	HS 680		0.288	0.043	<.0001
WBDC355	OUH602		0.173	0.047	0.0002

† Estimates of differences between least square means of inoculum treatments and non-inoculated controls

§Standard error of the differences

* Differences between inoculum treatment and non-inoculated control is not significant at $P < 0.05$

Appendix 5: Disease severity ratings, plant height and fresh seedling weight of putative M_3 mutants obtained from cultivar Lenetah

Progenitor M_2	M_3	DR[¶]	PH[†] (cm)	Fresh seedling wt (g)
M_Lenetah_1	M_Lenetah_1.1	2	21.0	0.689
	M_Lenetah_1.2	3	18.0	0.653
	M_Lenetah_1.3	2	20.5	0.737
	M_Lenetah_1.4	2	16.0	0.753
	M_Lenetah_1.5	2	18.0	0.775
	M_Lenetah_1.6	5	12.5	0.290
	M_Lenetah_1.7	5	15.0	0.392
	M_Lenetah_1.8	7	9.0	0.171
	M_Lenetah_1.9	5	16.0	0.654
	M_Lenetah_1.10	4	13.0	0.388
	M_Lenetah_1.11	6	18.0	0.374
	M_Lenetah_1.12	5	13.0	0.493
	M_Lenetah_1.13	8	10.0	0.197
	M_Lenetah_1.14	4	18.5	0.525
	M_Lenetah_1.15	4	17.0	0.671
M_Lenetah_2	M_Lenetah_2.1	3	20.0	0.621
	M_Lenetah_2.2	5	13.0	0.272
	M_Lenetah_2.3	4	18.0	0.580
	M_Lenetah_2.4	5	11.0	0.326
	M_Lenetah_2.5	5	17.0	0.393
	M_Lenetah_2.6	4	16.0	0.420
	M_Lenetah_2.7	4	16.0	0.500
	M_Lenetah_2.8	6	12.0	0.452
	M_Lenetah_2.9	8	13.0	0.258
	M_Lenetah_2.10	6	11.0	0.264
	M_Lenetah_2.11	6	18.0	0.537
	M_Lenetah_2.12	6	18.5	0.533
	M_Lenetah_2.13	6	21.5	0.563
	M_Lenetah_2.14	5	20.5	0.649
	M_Lenetah_2.15	8	12.0	0.294
	M_Lenetah_2.16	5	15.5	0.477

Appendix 5 continued……..

Progenitor M_2	M_3	DR[¶]	PH[†] (cm)	Fresh seedling wt (g)
	M_Lenetah_2.17	5	18.5	0.536
	M_Lenetah_2.18	7	20.0	0.414
	M_Lenetah_2.19	5	17.0	0.601
	M_Lenetah_2.20	5	19.0	0.594
	M_Lenetah_2.21	5	17.0	0.616
	M_Lenetah_2.22	7	16.5	0.446
	M_Lenetah_2.23	7	19.0	0.446
	M_Lenetah_2.24	5	17.5	0.573
	M_Lenetah_2.25	6	20.0	0.590
	M_Lenetah_2.26	8	13.0	0.266
	M_Lenetah_2.27	6	18.0	0.444
	M_Lenetah_2.28	6	18.5	0.596
	M_Lenetah_2.29	7	12.5	0.310
	M_Lenetah_2.30	5	15.5	0.574

[¶] Disease ratings

[†] Plant height

Appendix 6: Disease severity ratings, plant height and fresh seedling weight of putative M_3 mutants obtained from breeding line 05WA-316.99

Progenitor M_2	M_3	DR[¶]	PH[†] (cm)	Fresh seedling wt (g)
M_05WA_1	M_05WA_1.1	3	19.0	0.760
	M_05WA_1.2	2	21.5	0.683
	M_05WA_1.3	4	18.5	0.685
	M_05WA_1.4	4	16.5	0.478
	M_05WA_1.5	5	17.0	0.383
	M_05WA_1.6	5	16.0	0.600
	M_05WA_1.7	5	16.5	0.479
	M_05WA_1.8	4	14.0	0.350
	M_05WA_1.9	4	18.5	0.619
	M_05WA_1.10	4	14.5	0.514
	M_05WA_1.11	3	18.5	0.596
	M_05WA_1.12	4	15.0	0.487
	M_05WA_1.13	7	17.5	0.301
	M_05WA_1.14	5	18.5	0.345
	M_05WA_1.15	7	16.0	0.369
	M_05WA_1.16	5	12.5	0.317
	M_05WA_1.17	5	16.0	0.308
	M_05WA_1.18	5	16.5	0.386
	M_05WA_1.19	6	19.0	0.415
	M_05WA_1.20	5	14.0	0.308
	M_05WA_1.21	8	8.0	0.104
	M_05WA_1.22	7	15.5	0.328
	M_05WA_1.23	5	11.5	0.230
	M_05WA_1.24	5	14.5	0.252
	M_05WA_1.25	8	13.0	0.149
	M_05WA_1.26	8	11.0	0.178
	M_05WA_1.27	7	11.0	0.341
	M_05WA_1.28	6	15.0	0.186
	M_05WA_1.29	8	12.0	0.166
	M_05WA_1.30	7	13.0	0.122
	M_05WA_1.31	7	9.0	0.174
	M_05WA_1.32	6	11.5	0.220
	M_05WA_1.33	7	15.0	0.357
M_05WA_2	M_05WA_2.1	3	22.0	0.846
	M_05WA_2.2	3	19.0	0.807
	M_05WA_2.3	4	18.0	0.706
	M_05WA_2.4	5	19.0	0.708
	M_05WA_2.5	4	16.0	0.459

Appendix 6 continued…….

Progenitor M_2	M_3	DR[¶]	PH[†] (cm)	Fresh seedling wt (g)
	M_05WA_2.6	5	17.0	0.613
	M_05WA_2.7	4	20.0	0.670
	M_05WA_2.8	5	20.0	0.623
	M_05WA_2.9	5	15.5	0.530
	M_05WA_2.10	8	12.0	0.294
	M_05WA_2.11	5	17.5	0.580
	M_05WA_2.12	6	15.0	0.475
	M_05WA_2.13	5	16.5	0.732
	M_05WA_2.14	5	18.0	0.586
	M_05WA_2.15	6	17.5	0.393
	M_05WA_2.16	6	18.5	0.583
	M_05WA_2.17	5	18.0	0.668
	M_05WA_2.18	5	16.0	0.561
	M_05WA_2.19	5	18.0	0.578
	M_05WA_2.20	5	17.0	0.620
	M_05WA_2.21	5	19.0	0.607
	M_05WA_2.22	5	15.5	0.605
	M_05WA_2.23	5	16.0	0.624
	M_05WA_2.24	7	12.0	0.184
	M_05WA_2.25	5	18.0	0.578
	M_05WA_2.26	5	21.0	0.659
	M_05WA_2.27	5	18.0	0.596
	M_05WA_2.28	5	17.0	0.572
	M_05WA_2.29	5	16.5	0.384
	M_05WA_2.30	5	15.0	0.572
	M_05WA_2.31	5	19.0	0.505
	M_05WA_2.32	5	15.5	0.482
	M_05WA_2.33	5	15.0	0.428
	M_05WA_2.34	6	15.5	0.639
	M_05WA_2.35	6	17.0	0.467
	M_05WA_2.36	5	16.0	0.458
	M_05WA_2.37	4	18.0	0.573
	M_05WA_2.38	7	17.5	0.661
	M_05WA_2.39	5	16.0	0.323
	M_05WA_2.40	5	14.0	0.435

[¶] Disease ratings

[†] Plant height

Appendix 7: Disease severity ratings, plant height and fresh seedling weight of BC_1F_1 plants obtained from breeding line 05WA316.99

Progenitor M_3	BC_1F_1	DR[¶]	PH[†] (cm)	Fresh seedling wt (g)
Mutants as female ♀				
M_05WA_1.1	# 1	5	20.6	0.510
M_05WA_1.2	None	None	None	None
M_05WA_1.3	# 1	4	23.4	0.587
	# 2	3	20.0	0.711
M_05WA_2.1	None	None	None	None
M_05WA_2.2	# 1	7	12.5	0.186
	# 2	7	13.5	0.143
	# 3	7	14.0	0.208
	# 4	8	13.6	0.191
	# 5	7	15.0	0.207
	# 6	6	14.0	0.169
	# 7	7	10.4	0.144
	# 8	7	12.5	0.136
	# 9	7	11.5	0.154
	# 10	7	12.4	0.137
	# 11	8	11.1	0.157
	# 12	8	10.3	0.116
M_05WA_2.3	None	None	None	None
Mutants as male ♂				
M_05WA_1.1	# 1	3	22.4	0.678
	# 2	5	22.0	0.398
M_05WA_1.2	# 1	3	25.2	0.556
	# 2	3	24.0	0.579
	# 3	2	24.9	0.714
	# 4	3	20.0	0.657
M_05WA_1.3	# 1	3	23.5	0.777
M_05WA_2.1	# 1	6	17.3	0.240
	# 2	5	17.2	0.316
	# 3	6	14.2	0.226
	# 4	6	17.5	0.246
	# 5	7	10.6	0.103
	# 6	7	11.0	0.133
	# 7	8	12.0	0.142
	# 8	7	10.1	0.125
	# 9	8	11.0	0.126
	# 10	8	10.2	0.100
	# 11	8	10.4	0.085

Appendix 7 continued……

Progenitor M_3	BC_1F_1	DR[¶]	PH[†] (cm)	Fresh seedling wt (g)
M_05WA_2.2	# 1	4	17.6	0.329
	# 2	4	17.6	0.367
	# 3	6	17.4	0.325
	# 4	4	17.8	0.374
	# 5	4	17.1	0.352
	# 6	5	14.0	0.259
	# 7	4	19.0	0.409
	# 8	7	18.0	0.329
	# 9	7	16.0	0.369
	# 10	6	14.0	0.276
	# 11	6	16.8	0.260
	# 12	7	13.5	0.197
	# 13	4	19.0	0.426
	# 14	6	17.3	0.337
	# 15	5	14.2	0.300
	# 16	6	14.4	0.234
	# 17	5	15.5	0.225
	# 18	5	17.0	0.362
	# 19	5	15.5	0.323
	# 20	6	16.0	0.228
	# 21	8	11.4	0.120
	# 22	6	14.0	0.235
	# 23	7	16.0	0.279
	# 24	7	15.2	0.225
	# 25	7	14.0	0.192
	# 26	7	15.4	0.256
	# 27	8	13.4	0.164
	# 28	8	13.5	0.185
	# 29	7	13.3	0.191
	# 30	7	12.8	0.172
	# 31	8	12.2	0.137
	# 32	7	13.4	0.186
	# 33	7	14.1	0.182
	# 34	7	13.2	0.170
	# 35	8	13.4	0.131
M_05WA_2.3	# 1	7	15.0	0.190
	# 2	7	16.0	0.231
	# 3	6	15.1	0.211
	# 4	6	16.0	0.324

Appendix 7 continued……

Progenitor M_3	BC_1F_1	DR[¶]	PH[†] (cm)	Fresh seedling wt (g)
	# 5	6	15.0	0.190
	# 6	7	13.8	0.203
	# 7	7	15.0	0.215
	# 8	7	14.2	0.163
	# 9	7	14.0	0.189
	# 10	8	13.2	0.197
	# 11	7	15.0	0.193
	# 12	7	13.0	0.194
	# 13	8	14.6	0.209
	# 14	6	16.8	0.241
	# 15	7	13.0	0.145
	# 16	6	14.0	0.162
	# 17	7	12.5	0.146
	# 18	8	11.2	0.087
	# 19	8	9.5	0.102
	# 20	8	13.0	0.171

[¶] Disease ratings

[†] Plant height