T.R.
EGE UNIVERSITY
Graduate School of Applied and Natural Science

PHENOTYPIC ASSESSMENT OF RESISTANCE AGAINST SCALD DISEASE IN SPRING BARLEY GERMPLASM IN GREENHOUSE CONDITIONS

MSc THESIS

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Department of Seed Science and Technology

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GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

DECLARATION

I hereby declare that this thesis study entitled as "**Phenotypic assessment of resistance against scald disease in spring barley germplasm in greenhouse conditions**" represents my own work which has been done after registration for the degree of M.Sc. at Ege University and has not been previously included in a thesis or dissertation submitted to this or any other institution for a degree, diploma, or other qualifications. I also declare that all the information in this document has been obtained and presented in accordance with Ege University academic rules and regulations as well as ethical conduct. Lastly, I declare that, as required by these rules and conduct, I have fully cited and referenced all material and results, which are not original to this work.

10/08/2022

Su Myat NOE

ÖZET

SERA KOŞULLARINDA YAZLIK ARPA GERMPLASMINDA ARPA YAPRAK LEKESİ HASTALIĞINA KARŞI DAYANIKLILIĞIN FENOTİPİK DEĞERLENDİRİLMESİ

NOE, Su Myat

Yüksek Lisans Tezi, Tohumluk Bilimi ve Teknolojisi Anabilim Dalı
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Bu çalışmada, kontrollü koşullarda *Rhynchosporium commune*'nin neden olduğu arpa yaprak lekesi hastalığına karşı dayanıklı yazlık arpa genotiplerinin belirlenmesi ve değerlendirilmesi yapılmıştır. Deneme, İşveç Tarım Bilimleri Üniversitesi (SLU), Bitki Islahı Bölümünde iklimlendirilmiş Biotron odasında gerçekleştirilmiştir. Çalışmada, Nordgen Gen Bankasından temin edilen yaklaşık 288 arpa genotipi ve dört ticari çeşit (Freja, Ingrid, Laureate ve RGT Planet) kullanılmıştır. Deneme, çok sayıda genotip için uygun olan Augmented tesadüf blokları deneme desenine göre kurulmuştur. Genotipler denemede iki set (tekerrür) halinde kullanılmıştır. Her bir tekerrürde 14 blok yer almış ve bloklarda arpa genotipleri rastgele dağıtılmıştır. Her blokta dört kontrol genotip yer almıştır. Genotiplerin ekimine paralel olarak, SLU Bitki Islahı Bölümünün laboratuvarında inokulum hazırlığı (miselyum büyümesi için CZV8CYM ortamında ve sporülasyon için WGA ortamında *R. commune*'nin kültüre alınması) gerçekleştirilmiştir. *R. commune*'nin sporülasyon oranı 1.35 x 106/ml olarak belirlenmiş ve hemositometre ile sayarak doğrulanmıştır. Bitkilerin ikinci ve üçüncü yaprakları geliştiğinde inokülasyon yapılmıştır. Hastalık skorlaması, inoküle edilen ikinci ve üçüncü yapraklardaki enfeksiyona dayalı olarak üç gün aralıklarla (inokülasyondan sırasıyla 11, 14 ve 17 gün sonra) üç kez yapılmıştır.

Elde edilen skorlama verileri analiz edilip ortalama değerler RStudio programında Augmented tesadüf blokları deneme deseni paketi kullanılarak

düzeltilmiştir. ANOVA sonucuna göre, birinci tekerrürdeki ikinci skorlama zamanı (14. dün) dışında, her iki tekerrürde de test edilen 288 genotip arasındaki skorlama sonuçları önemli saptanmış ve genotipler arasında arpa yaprak lekesi hastalığına karşı dayanıklılık seviyesi farklı bulunmuştur. Ancak, test edilen genotipler ile kontrol genotipler arasında dayanıklılık açısından karşılaştırma yapıldığında önemli bir fark saptanmamıştır. Bu sonuç, kontrol çeşitlerin hastalığa vermiş oldukları farklı tepkilerden olabilir. Kontrol çeşitlerden Laureate ve RGT Planet hastalığa dayanıklı, Freja ve Ingrid hassas olarak bilinmektedir. Elde edilen düzeltilmiş ortalamalar, hastalığın zaman içindeki ilerlemesini hesaplamak için gerekli ve etkili bir yöntem olan hastalık gelişim eğrisi altındaki alan (AUDPC) için analiz edilmiştir. Hastalık gelişim eğrisi (AUDPC) sonucuna göre, en düşük AUDPC değerine sahip ilk beş genotip, birinci tekerrürde Lofa, Solar, ST_13947, Akka ve Rika ve ikinci tekerrürde Lofa, Solar, Pongo, Akka ve ST_13947 olmuştur.

Buna karşın, en yüksek AUDPC değerine sahip ilk beş genotip birinci tekerrürde BOMI, Drost A, Vega, Pallas ve Freja ve ikinci tekerrürde Herta, Foma, PR_9275, DS 9770_4 ve DS 10060_4 olarak belirlenmiştir. Test edilen genotiplerin en iyi lineer yansız tahminini (BLUP) belirlemek için iki tekerrür birlikte analiz edildiğinde, en düşük AUDPC değerine sahip olanlar Lofa, Solar, Akka, St_13947 ve Pongo genotipleri olmuştur. Buradaki ilk dört genotip birinci tekerrür sonucu ile aynı genotiplerdir. Test edilen genotiplerin geniş anlamda kalıtım derecesi 0,725 olarak belirlenmiştir. Elde edilen bu değer, önceki literatür çalışmalarında belirlenen 0,20-0,97 aralığındaki geniş anlamda kalıtım derecesi değerlerinden yüksek olarak bulunmuştur. Bu çalışma, test edilen genotiplerde zaman içinde arpa yaprak lekesi hastalığının ilerlemesini fenotipleyerek dayanıklı genotiplerin belirlenmesi hakkında temel bilgiler sağlamıştır. Bu genotiplerin hastalığa karşı dayanıklılığını, çok lokasyonlu denemeler yaparak ve hastalık ilerlemesini fenotipleyerek veya QTL analizini dahil ederek dayanıklılık genlerinin varlığını belirleyerek doğrulamaya ihtiyaç vardır. Böylelikle arpa yaprak lekesi hastalığına karşı dayanıklı arpa genotiplerinin ıslah programını hızlandırmak mümkün olabilir.

Anahtar Kelimeler: Yazlık arpa, *Rhynchosporium commune*, arpa yaprak lekesi hastalığı, kalıtım derecesi, BLUP.

ABSTRACT

PHENOTYPIC ASSESSMENT OF RESISTANCE AGAINST SCALD DISEASE IN SPRING BARLEY GERMPLASM IN GREENHOUSE CONDITIONS

NOE, Su Myat

Msc Thesis, Seed Science and Technology

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This study evaluated and identified the resistant spring barley genotypes against scald disease caused by *Rhynchosporium commune* under a controlled environment. The experiment was conducted in the climatized chamber of Biotron at the Department of Plant Breeding, SLU, Alnarp, Sweden. The experiment included about 288 barley-tested genotypes by Nordgen genebank and four commercial cultivars (Freja, Ingrid, Laureate, and RGT Planet). The experimental design used in this study was Augmented RCBD, which was suitable for an experiment with large numbers of treatments (genotype). In this experiment, three sets (replications) of genotypes were included. Each set included 14 blocks where the tested barley genotypes were assigned randomly among those blocks and four checks in every block. In parallel to seed sowing, inoculum preparation (culturing of *R. commune* on CZV8CYM media for mycelium growth and WGA media for sporulation) was carried out in the laboratory of the Department of Plant Breeding, SLU. The rate of *R. commune* sporulation was 1.35 x 10^6/ml and confirmed the result by counting with a hemocytometer. Inoculation was done when the second and third leaves of the plant developed. Disease scoring was done three times at three-day intervals (11DAI, 14 DAI, and 17DAI, respectively) based on infection on the inoculated second and third leaves.

The collected scoring data were analyzed, and the mean values were adjusted using augmentedRCBD packing in RStudio software. According to the ANOVA

result, except for the second scoring time (14 DAI) in augmented set 1, scoring results among 288 tested genotypes were significant in both augmented sets meaning the resistance level among these genotypes against scald disease was different. However, there was no significant difference when the tested genotypes and checks were compared for the resistance. It might be due to the different response levels of check cultivars where Laureate and RGT Planet were known to be resistant. At the same time, Freja and Ingrid were susceptible to scald disease. The resulting adjusted means (scoring) were further analyzed for the area under the disease response curve (AUDPC), an essential and effective method to calculate disease progressiveness over time. According to the AUDPC result, the top five genotypes with the lowest AUDPC were Lofa, Solar, ST-13947, Akka, and Rika for set 1 and Lofa, Solar, Pongo, Akka, and ST-13947 for set 2.

Conversely, the top five genotypes with the highest AUDPC were BOMI, Drost A, Vega, Pallas, and Freja in set 1 and Herta, Foma, PR-9275, DS 9770-4, and DS 10060-4 in set 2. When the two sets were jointly analyzed to determine the best linear unbiased prediction (BLUP) of the tested genotypes, the ones with the lowest AUDPC values were Lofa, Solar, Akka, St-13947, and Pongo, of which the first four genotypes resulted in the combined analysis were identical to those in the individual trial set. The broad-sensed heritability of the tested genotypes was 0.725, which could be considered a high-level estimation based on the previous literature studies obtaining a broad-sensed heritability within 0.20-0.97. This study provided basic information about identifying resistant genotypes by phenotyping the disease progression of scald in the tested genotypes over time. It would need additional steps to confirm the resistance of these genotypes against scald disease by doing multilocational trials and phenotyping disease progress or incorporating QTL analysis by identifying the presence of resistant genes and speeding up the breeding program resistant barley genotypes against scald disease.

Keywords: Spring barley, *Rhynchosporium commune*, scald, heritability, BLUP.

PREFACE

Barley is one of the major cereals for humans and has been many decades benefiting humankind as well. Ancient humans grew barley mainly for their diet, but in today's era, the purpose of growing barley has been changed and mainly focuses on animal feed and malting. Depending on the variety, barley can be grown in both winter and spring, of which spring barley is a short duration. In contrast, winter barley is a long duration and requires vernalization for flowering and fruit sets. In Sweden, spring barley is much more interested because, in winter, farmers grow winter wheat more.

Altogether with the wide adaptability of barley, it is undeniable that it has a different range of biotic and abiotic stresses. Among different biotic stresses, foliar diseases such as *Septoria tritici* blotch and tan spot are serious diseases to winter barley, while net blotch and scald diseases were recorded as high impacted for spring barley. Scald is a fungal disease caused by the *Rhynchosporium commune.* Its damage rate in barley production reached 40% of yield loss and grain quality reduction, significantly when susceptible cultivars are grown. Moreover, *R. commune* is one of the pathogens with a high rate of variation, and intensive application of fungicides exerts an intense selection pressure leading to the breakdown of resistance of the host plant.

Although discovering a couple of resistance genes against scald disease and some genotypes such as RGT Planet, known to be resistant to scald disease, are available in the market, no one can guarantee how the resistance will last long. The plant breeders must develop barley cultivars with a more robust and durable resistance against scald disease for the world population. With this purpose, the experiment was carried out as one of the pre-breeding stages to evaluate and identify spring barley genotypes resistant to the scald disease. The phenotypic results from the experiment will be helpful for future breeding programs in identifying resistance genes/QTLs against scald disease.

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1. INTRODUCTION

Barley (*Hordeum vulgare* L.) is an annual cereal belonging to the Poaceae family. Although barley can be grown in various environments, it is primarily cultivated in temperate regions. Barley occupies the fourth position in cultivation among the five most essential cereal crops worldwide (Akar et al., 2004). According to archaeological evidence, barley was domesticated from its ancestor, the wild barley (*Hordeum spontaneum*), in the fertile crescent region (Hughes et al., 2019). Initially, Neolithic people sought to cultivate it for their consumption, whereas the current production is primarily intended for animal feed and beverage production (Brown et al., 2009). Depending on its growth habit, barley varieties can be classified as spring or winter genotypes, with winter barley requiring vernalization for flowering and seed setting. Generally, barley spike (head) has three spikelets, one in the center and two on the laterals, attached alternately to the rachis node. While the central spikelet is the only fertile spikelet in wild barley, all spikelets mature into grains in cultivated barley (Magness et al., 1971). Based on the vantage point of the spike and the presence of fertile grain, barley is classified as either two-rowed or six-rowed, with two-rowed barley referring to wild barley and six-rowed barley referring to cultivated barley (Komatsuda et al., 2007). Sweden's most commonly grown spring barley is the two-rowed type for malting because of its high sugar content (Tillgren, 2021). In 2020-2021, the world produced approximately 160.53 million metric tons of barley, with the European Union accounting for 52.75 million metric tons and leading the global production, followed by Russia and Australia, which produced 17.5 and 13 million metric tons, respectively (Shahbandeh, 2022). Winter wheat and spring barley are the principal cereals produced in Sweden to narrow the production focus to Sweden (Buntaran et al., 2020).

Crop yield is undeniably an essential objective of plant breeding programs; however, significant abiotic and biotic constraints prohibit it. Decades of biotic stress such as drought, temperature increase, salinity, and nutrient deficiency have impacted barley production in European nations (Moore and Lobell, 2015). Alternatively, mainly plant diseases, abiotic stresses significantly hinder barley production (Singh et al., 2019). There is a wide range of pathogens in barley,

including bacteria, fungi, nematodes, and viruses. It is generally due to the broad adaptability of barley, which generates a diverse array of pathogens (Asif and Kamran, 2012). Among the pathogens affecting barley, *Rhynchosporium commune*, which causes scald or leaf blotch disease in barley, is a significantly and economically important disease in European barley production (Avrova and Knogge, 2012) because it can reduce barley yield by up to 40 percent, particularly in susceptible cultivars (Paulitz and Steffenson, 2011). Scald disease is present in all barley-growing regions of the world, including northern and central Europe, Asia, Africa, the Americas, Australia, and New Zealand (Brunner et al., 2007; Robbertse et al., 2001).

Although barley was domesticated approximately 10,000 years ago, *R. commune* was not present at that time but later emerged in Northern Europe by switching its host from wild grass to cultivated barley (Penselin et al., 2016). Several researchers have reported multiple QTLs on different chromosomes for scald resistance in barley, including Rrs1 on 3H, Rrs2 on 7H, and Rrs13 on 6H in various barley genotypes (Abbott et al., 1991; Bryner, 1957; Dyck and Schaller, 1961). However, it was later discovered that the identified QTLs with the same resistance were overlapping among the tested genotypes across multiple studies, resulting in a disillusioning result regarding identifying resistance genes in barley (Weibull et al., 2003). Therefore, it is essential to investigate resistant genes to expedite breeding one of the world's most important crops, barley. To this end, evaluating disease resistance in as many genotypes as possible would provide a solid foundation for plant breeders to continue the genomic study of scald resistance in barley.

Here, a diverse panel of 288 barley genotypes available at Nordgen were tested for scald resistance by artificially inoculating the plants with a virulent isolate of *R. commune*. We hypothesized that variations of response to scald disease should follow the diversity of the tested genotypes while enabling the discovery of highly resistant ones under a controlled environment. To test this hypothesis, the following research objectives were established.

1) To culture and inoculate *R. commune* to the tested barley genotypes
2) To evaluate *R. commune* resistance in the tested barley genotypes
3) To identify the most resistant genotypes against *R. commune* for use in future breeding programs

2. LITERATURE REVIEW

2.1 Barley: One of The First Domesticated Crops By Humans

Barley (*Hordeum vulgare* L.) is an annual grass that belongs to the family Poaceae. It is listed under the genus *Hordeum* altogether with other thirty-two species which possess similar morphological characteristics, such as bearing triplet spikelets at each rachis node (Bothmer et al., 1995). However, the *Hordeum* is distributed worldwide (mostly abundantly in temperate regions). Accordingly, their centers of origin are recognized as southern South America, western North America, the Mediterranean, and Central Asia (Bothmer et al., 1995). It is a diploid plant species with fewer chromosomes (2n=14) but a large genome. The wild barley cultivar (*Hordeum spontaneum*) is considered the immediate progenitor of the cultivated barley (both two-rowed and six-rowed barley where the latter was developed because of more than one mutation (Bothmer et al., 1995). Traits of barley important for early domestication are brittleness of rachis, kernel row type (two-rowed or six-rowed), covered and naked kernels, dormancy, growth habit (spring or winter), productivity and quality traits such as grain color, abiotic resistance such as salinity tolerance, and disease resistance.

Barley is a nutritious cereal containing moderate protein content, the highest percentage of carbohydrates, and dietary fiber, known as beta-glucan-this fiber helps reduce LDL-cholesterol (LDL-C) (Keenan et al., 2007). Being a healthy crop, the Romans, mainly the gladiators, consumed barley as their primary diet together with beans. As time went by, the utilization of barley changed significantly from human daily dietary to animal feed and malting purposes (Kumari and Kotecha, 2015; Lösch et al., 2014). According to Figure 2.1, a glance at the ten years from 2012-to 2022 showed that animal feed production primarily occupied the world's barley demand. Barley is also considered a substitutional crop to maize where it cannot be grown well (Zhou, 2010).

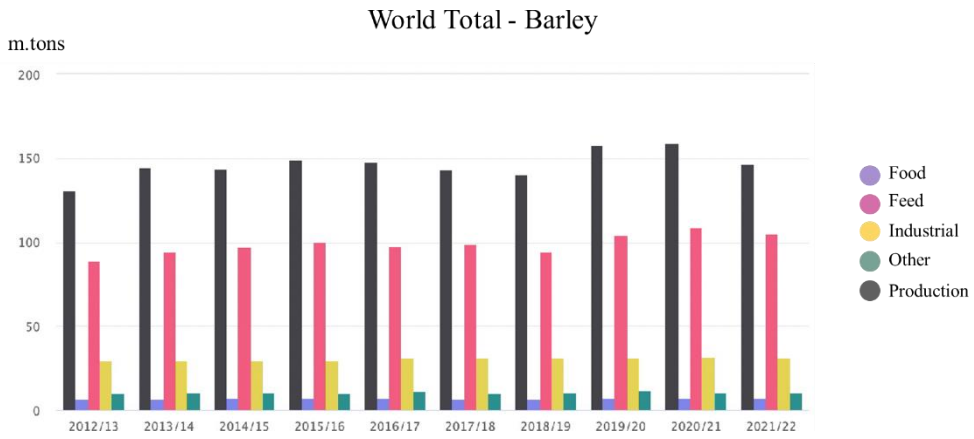

Figure 2.1. World barley production within ten years (2012-2022) referenced from International Grains Council

Approximately 400,000 barley accessions are conserved worldwide where International Center for Agricultural Research in the Dry Areas (ICARDA) maintains about 32,000 barley accessions (8% landraces and 7% wild relatives) while genebanks in Australia, Brazil, Canada, China (CAAS), Ethiopia, Germany (IPK), Japan, Mexico, Russian Federation, South Korea, Sweden, UK and USA (ARS) conserve more than 10000 barley accessions individually (ICARDA, 2016).

2.2 Barley Cultivation in Nordic Countries

Barley has been cultivated in Scandinavian countries for over 6000 years (Vorren, 2005). Sweden is one of those countries, and its significant crops are cereals such as wheat, barley, oats, and pasture. Barley is grown all over the country, but the majority is in the northern part, and the most common cultivated barley is spring two-rowed barley. The production from northern part contributes thirty percent of total country production. In 2020, Sweden produced approximately 1.5 million tons of barley with an average yield in 2020 was 51,715 tons per hectare. However, data of recent decades show a significant fluctuation in barley grain yield from 1970 to 2000 (Knoema Data Hub).

2.3 *Rhynchosporium Commune*: A Causal Pathogen to Barley Scald Disease

Initially, the causal pathogen of scald disease was assumed to be *R. secalis* which infects rye, triticale, and barley. Later, it was discovered that the pathogen is host-specific, with the strain infecting barley being a distinct species known as *R. commune* (Zhang et al., 2020). *R. commune* is a hemibiotroph (a mixed form of biotrophic and necrotrophic) that acts as a parasite by invading the host living tissues during the initial infection period (biotrophic manner) and finally killing the host cells along with their intrusion (necrotrophic manner) (Divon and Fluhr, 2007). Although this pathogen has been known to reproduce asexually for an extended period, a recent study established that it also has sexual reproductive mechanisms despite the absence of ascocarp (the sexual fruiting bodies) footage (Zhang et al., 2020). *R. commune* can overwinter on crop residues, seeds, and soil, with debris and seeds serving as the primary source of inoculum and sporulation. Also, rain splash contributes to secondary inoculum, which significantly affects conidia dispersal to neighboring plants (Zhang et al., 2020).

Conidia of *R. commune* is two-celled and beak-shaped in structure, and its fungal growth in barley occurs in four phases. First, the conidia germinate after 12 hours of inoculation/infection by the development of germ tubes and subsequently followed by the formation of appressoria at the tip of the germ tubes. Approximately 24 hours after inoculation/infection, the penetration peg penetrates the host cell cuticle as the second phase of fungal growth. Then a thin fungal hypha grows intracellularly and longitudinally alongside the leaf surface (no mycelium growth at this moment). During the fourth phase (approximately ten days after inoculation/infection), the mesophyll cells collapse because of the massive intercellular growth of mycelium (Ayesu-Offei and Clare, 1970; Zhan et al., 2008). At this stage, the greyish water-soaked lesions can be seen on the leaf surface, and these lesions later turn into chloric and necrotic ones gradually (Jackson, 1997). Afterward, the final appearance of scald disease with pale or faded water-soaked lesions surrounded by a deep-brown colored margin is visible on the leaf blade (Lehnackers and Knogge, 1990). Additionally, the disease can develop without visible symptoms while the fungus is growing inside the host plant tissue.

Consequently, the pathogen can be transmitted to harvested seeds (Atkins et al., 2010; Walters et al., 2012). While rain splash is the primary disease transmitter for nearby plants, the infected seeds can be a source for generation by generation and long-distance transmission of the disease (Fountaine et al., 2010; Stefansson et al., 2012).

The most widely used practices for plant disease management are fungicide application and growing resistant cultivars solely or an integrated approach. Nonetheless, both conceptually apply selection pressure to pathogens, by which the pathogen can overcome fungicide treatment and host plant resistance. In the case of fungicides, pathogens develop resistance to them, whereas to circumvent the plant defense system, they modify their avirulence gene product (a protein) to one that is not recognized by the plant receptor located on the plasma membrane (Brown, 2015; Sacristán and García-Arenal, 2008). The evolution rate of *R. commune* is relatively high, and it can easily be adaptable to different environmental conditions (Habgood, 1973; Jackson and Webster, 1976; Q. Zhang et al., 1992). The isolates taken from the same lesion (sample area) are different in color, sporulation, germination, and fungal aggressiveness (virulence). (Ali et al., 1976; Brown, 1985).

2.4 Genebank Germplasms: A Genetic Resource For Developing Resistance Cultivars

Up to 2019, there are 1,750 genebanks worldwide thatch that conserves 7.5 million germplasm including landraces, wild relatives, and mutants maintained in different forms of conservation such as seed, other plant parts including shoots, and pollens (CGIAR, 2019). It is undoubtful that landraces and wild landraces have the tolerance and resistance to biotic stresses. In wheat, about 44% of leaf rust disease resistance genes (*Lr* genes) in wheat have been discovered from its wild relatives conserved in genbanks (Fatima et al., 2020). Moreover, about 21 resistance (major) genes related to tritici blotch (STB) resistance in wheat were identified, and among several QTLs discovered previously, four putative QTLs on chromosome 3A, 5A, 4B, and 5B were reported from Ethiopian Durum Wheat Landraces (Kidane et al., 2017). Furthermore, two rice resistance genes, *Pi-ta* and *Pi54* for blast disease were

discovered in two landraces of Indica rice, Tetep and Tadukan (Mahesh et al., 2016).

Barley is attacked by several pathogens among of which that can cause the highest yield losses is *Pyrenophora tritici-teres*, the causal agent of net form net blotch (NFNB) disease. NFNB is a significant disease for barley in its production areas. The use of land races and commercial cultivars from barley center of origin has aided in the identification of four new QTLs located on chromosome 3H, 5H, and 6H associated with resistance against NFNB disease (Novakazi et al., 2019). On the other hand, several resistance genes had been mapped from the cultivated barley species (*Rrs1* on 3H and *Rrs2* on 7H) as well as from wild *Hordeum* species (*Rrs12* on 7H and *Rrs13* on 6H) against scald (Abbott et al., 1991, 1995; Hanemann et al., 2009; Hofmann et al., 2013). The most recent identification of the scald resistance gene was *Rrs18* on chromosome 6H by Hofmann (2014). Although different resistance (major) genes have been discovered for scald disease, the gene-for-gene interaction between the avirulence proteins (effectors) of the pathogen (*R. commune*) and the resistance R genes of the host plant (barley) always leads to the evolutionary mechanism resulting in the overcoming of plant defense system by the pathogen in a short period (Barua et al., 1993). For example, necrosis-inducing peptides 1 (NIP1) protein produced by *R. commune* is recognized by the *Rrs1* on the 3H chromosome of the scald resistance barley cultivars and for. It forces open to modifying its function of producing NIP1 effector by mutating or losing it (Rohe et al., 1995). The most appropriate way to deal with it is the pyramiding of major genes and QTLs with partial effects against creating a more durable resistance to scald disease (Walters et al., 2012). Therefore, the exploitation of qualitative and qualitative resistance genes from various genetic resources such as germplasms concerning barley scald disease is crucial.

2.5 The Use of Area Under Disease Progress Curve (AUDPC) In Phenotypic Selection

Plant epidemiologists use the disease progression curve to illustrate the progression of the disease in the host plant over time (and sometimes space) and evaluate the host's resistance (Luke and Berger, 1982; Pennypacker et al., 1980). In

the 1980s, plant pathologists used simple descriptive models to visualize disease progression over the tested time frame, for instance, a comparison study of rust severity in different oat cultivars (slow-rusting and fast-rusting ones) with a disease scoring interval of 6-7 days (Luke and Berger, 1982). (Campbell and Madden, 1990) introduced the trapezoidal method for calculating the area under the disease response curve (AUDPC). This method typically determines the disease severity by summing the average disease intensity between the closest scoring time points, beginning with the initial scoring, and ending with the final scoring. Many experiments, including field research as well as greenhouse ones, were conducted to study the quantitative resistance in many cereal crops. For example, the study of *Septoria tritici* epidemics in winter wheat (Paraschivu and Cotuna, 2013), assessment of quantitative resistance against leaf rust in winter rye hybrids (Miedaner and Sperling, 1995), evaluation of maize inbred lines against grey leaf spot disease (Saghai Maroof et al., 1993) were carried out. In barley, studies in which the analysis of disease severity distribution among panels of genotypes was examined using the AUDPC approach. The assessment of Tunisian barley against net blotch disease (Cherif et al., 2010), evaluation of adult plant resistance in two-rowed and six-rowed barley genotypes against spot blotch disease, and comparison of the yellow rust disease resistance among the naked barley landraces (Visioni et al., 2020).

2.6 Best Linear Unbiased Prediction (BLUP): A Valuable Tool To Predict The Breeding Values

Plant breeders adopted the mixed model equation developed by C. R. Henderson in 1948, which was initially applied to animal breeding to select the most promising progeny among the tested ones. The linear mixed model allows for the evaluation of both fixed effects by best linear unbiased estimation (BLUE) and random effects by best linear unbiased prediction (BLUP) individually (Henderson, 1975). Although among these two evaluations, BLUP-based selection returns a more accurate selection result than BLUE-based one (Copas, 1983) because of the shrinkage properties of BLUP, which drags the above and below-means to the mean value (Hill Jr. and Rosenberger, 1985) and later BLUP was widely applied in plant breeding (Piepho et al., 2008). Additionally, BLUP is beneficial in analyzing

unbalanced data, i.e., missing plants due to poor germination, seedling damage, or rodents, especially under field conditions. BLUP allows the calculation of the effect of the covariate factor on the primary variable (Alvarado et al., 2015). This statement was further supported by (Robinson, 1991) that BLUP was helpful in plant varietal selection because of its capacity to return the predicted values after adjusting with the mean value and ranking the genotypes accordingly. Much research has been carried out to select the most superior genotypes among the tested ones based on BLUP-based information. One of those was research conducted to select the best genotypes from two ornamental species (Molenaar et al., 2018). Moreover, a simultaneous combination of AMMI (additive main-effects and multiplicative interaction) and BLUP recommended high-yielding barley genotypes (Verma et al., 2021).

3. MATERIALS AND METHODS

3.1 Experimental Design and Materials

In this study, 288 spring barley genotypes from the Nordic gene bank Nordgen together with 4 control genotypes were grown in the cultivation unit (Biotron) of the department of plant breeding, Swedish University of Agricultural Sciences (SLU), Alnarp, Sweden (Figure 3.1). The biotron provides growth environment that allows to grow plants in precisely climate-controlled chambers for different parameters such as light, temperature and humidity.

Figure 3.1. Experimental site location

The genotypes were arranged in Augmented Randomized Complete Block Design (AugmentedRCBD) with two replicates using the Agricolae package in RStudio statistical software. According to this design, there were 14 blocks in each replicate. Additionally, the tested genotypes were assigned randomly among the 14 blocks in which each block contained 19-21 tested barley genotypes altogether with four control genotypes (Figure 3.2). One day before sowing, 8x8x9 cm plastic pots were filled with compost. Eight seeds for tested genotypes and six seeds for checks were sown on 11 March 2022. Seven days after sowing, thinning was done by

leaving two seedlings per pot. After thinning, an equal amount of mono potassium phosphate (KH_2PO_4) solution (diluting 15g of KH_2PO_4 in 1000 ml of water) was applied to all blocks.

Figure 3.2. Experimental layout using Augmented Randomized Complete Block Design

3.2 Inoculum Preparation, Isolation, and Inoculation of *Rhynchosporium Commune*

The protocol applied in this experiment to isolate and inoculate *R. commune* is prepared/modified by Mustafa Zakieh, Aakash Chawade Lab, Department of Plant Breeding, SLU, Alnarp, Sweden.

3.3 *Rhynchosporium Commune* Inoculum Preparation

To obtain *R. commune* inoculum, three types of culture media were required. A simple water agar media was used as an initial step for preserving *R. commune* infected leaf samples. For the second step of *R. commune* mycelium growth, a specific type of media, CZV8CYM, was used. For the last step of *R. commune* inoculum preparation, wheat germ agar media was used for *R. commune* sporulation.

3.3.1 Environmental sample incubation on water agar media

The first media type was the water agar to incubate the barley leaf samples infected with scald. These samples were taken from the barley production fields in the Southern part of Sweden near Ystad. The collected scald infected leaf cuts were sterilized with 0.5% sodium hypochlorite approximately for 2-3 minutes and then rinsed three times for 5 minutes with sterile distilled water to remove sodium hypochlorite. Then the leaf cuts were moved to 90 mm Petri dishes containing water agar (agar and distilled water) and rifampicin antibiotic (100 µg/L) to prevent bacterial infection to the culture media and left to incubate at room temperature for two weeks. The *R. commune* mycelium grown on water agar media was provided by the Department of Plant Breeding, SLU. The second and third types of culture media were prepared for *R. commune* mycelium growth and sporulation during this experiment.

3.3.2 *R. commune* mycelium growth and occasional sporulation CZV8CYM media

In order to prepare the mycelium growing media for R. commune, the following ingredients were added.

Table 3.1. Ingredients to prepare CZCVCYM media

Oxid Czapek Dox	56 g
Bacto agar	10 g
V8 juice	200 ml
Calcium carbonate	4 g
Yeast extract	1 g
Malt extract	1 g
Distilled water	800 ml

These ingredients were mixed thoroughly, resulting in a 1000 ml CZV8CYM solution, and then it was autoclaved for about 20 minutes. Then the autoclaved solution was added to 90 mm Petri dishes, and the dishes were cooled down until the added solution was completely solidified.

Two weeks old *R. commune* mycelium were transferred to CZV8CYM media and incubated in the dark (additionally covering the Petri dishes with aluminum foil) at 17-18 °C for two weeks until the growth of mycelium reached around 1-3 cm in diameter (Figure 3.3).

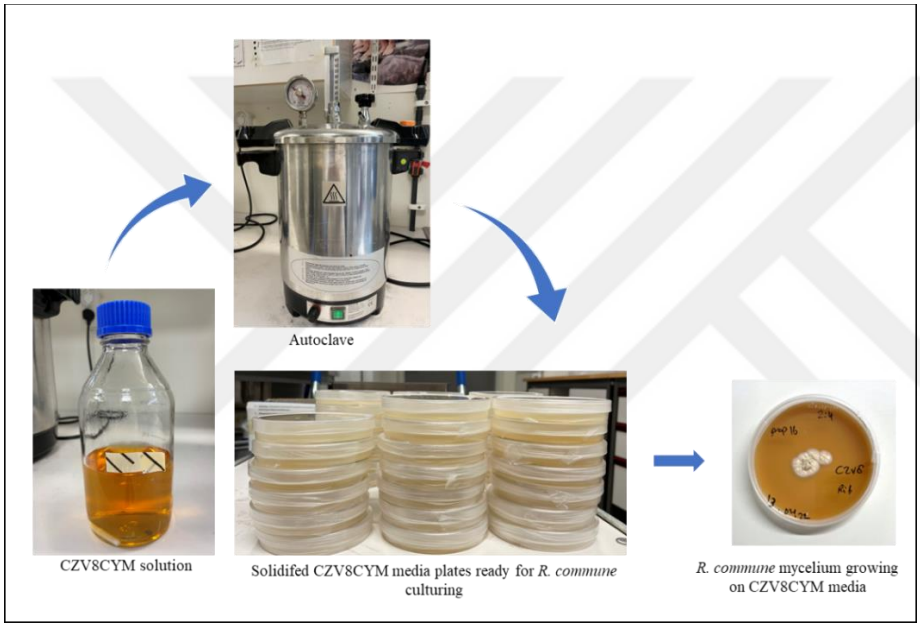

Figure 3.3. CZV8CYM media preparation and culturing of *R. commune* for mycelium growth

3.3.3 *R. commune* sporulation on wheat germ agar media

In order to induce fungal sporulation or for the induction of spore formation, fungal plugs were taken from two-week-old mycelium of *R. commune* and transferred to WGA.

The main ingredients were wheat germ and agar to prepare wheat germ agar. The ratio used in this experiment was 10% of the weight/volume of wheat germ agar to regular tap water. About 100 g of wheat germ agar was added to 1000 ml of

tap water, and the mixture was boiled for 10 minutes and simmered for 20 minutes. Then the mixture was cooled down at room temperature and centrifuged at 2000 rpm for 20 minutes. Then the resulting supernatants were collected and restored to the original volume (1000 ml) by adding tap water, then autoclaving the suspension at 121 ℃ for 20 minutes. The suspension was cooled down at room temperature, and then agar was added to the suspension with a ratio of 15g per liter of water. The mixture was warmed for 5 minutes, added to 90 mm Petri dishes, and cooled down. The harvested *R. commune* mycelium was transferred to the wheat germ agar containing Petri dishes and stored in the darkroom at 17-18 ℃ for two weeks for sporulation (Figure 3.4).

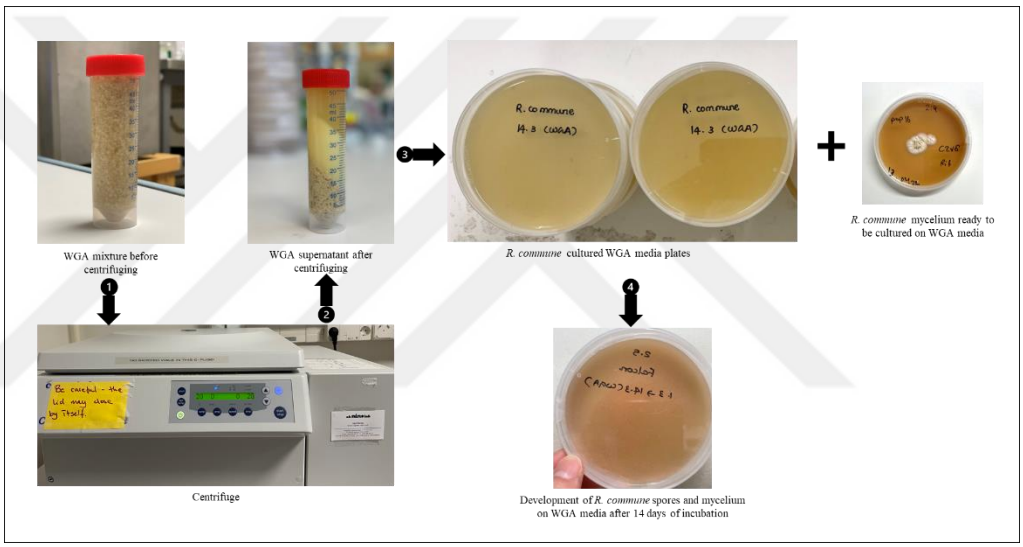

Figure 3.4. WGA media preparation and culturing of *R. commune* for sporulation

3.4 Harvesting of *R. Commune* Conidia and Inoculating To Tested Barley Genotypes

To collect the well-grown *R. commune* conidia in wheat germ agar media, 15-20 ml of deionized distilled water was added to individual Petri dishes and brushed thoroughly by applying a moderate hand force to collect the conidia as much as possible, resulting in 1000 ml of spore suspension. Then the conidia were counted using a hemocytometer, and the number of *R. commune* conidia in the suspension was adjusted to 1.35×10^6 conidia/ml of suspension (Figure 3.5).

Thereafter the original suspension was diluted to 6000 ml by adding distilled water, which was sufficient to inoculate barley plants in 28 blocks. The inoculation was carried out by hand-spraying to the whole plant level when it developed at three leaf stage (Figure 3.6). Immediately after inoculation, the plants were kept under dark conditions by covering the trolleys with transparent plastic sheets and aluminum sheets and maintaining the chamber in a fully humidified condition for 72 hours at 16-17 ℃ (Figure 3.7). Three days after inoculation, the covers were removed and kept in the greenhouse chamber with a constant temperature of 17 ℃ and 16/8 hours of light/dark cycle with 75% relative humidity (rh).

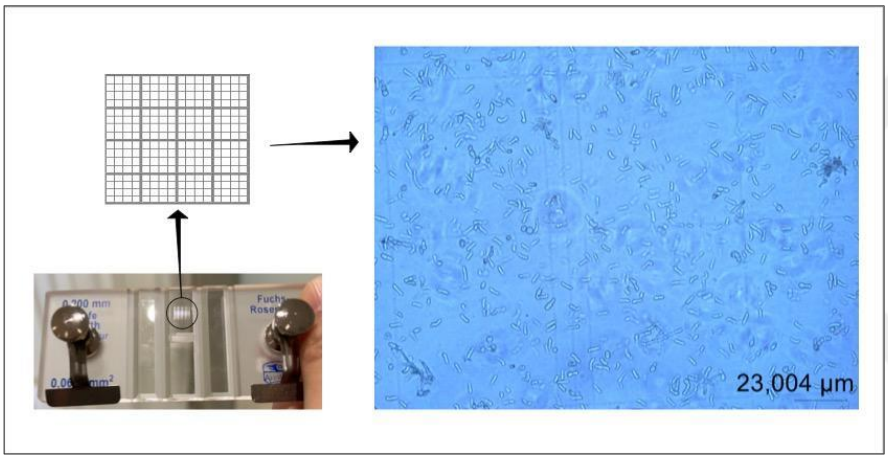

Figure 3.5. Counting *R. commune* spores using a hemocytometer

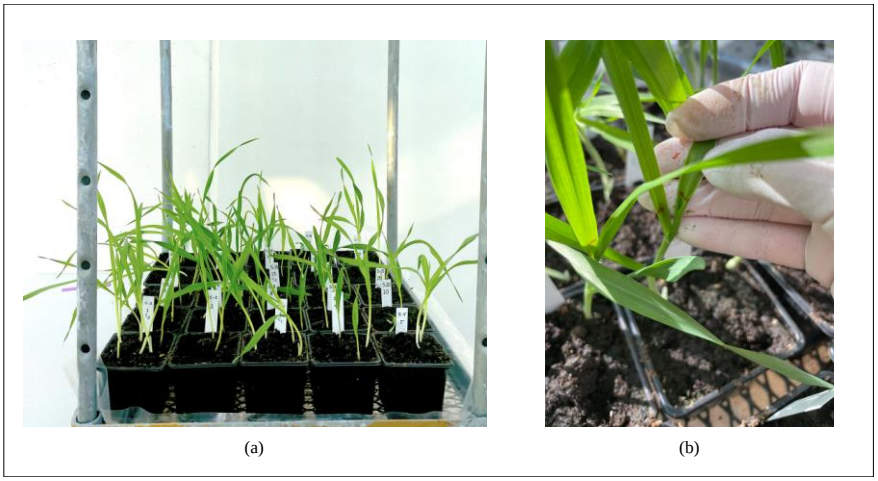

Figure 3.6. Barley seedlings which are ready to be inoculated (a) second and third leaves developing stages of barley seedling (b) marked second and third leaves for disease scoring

3.5 Assessment of Scald Disease Incidence

Disease scoring was carried out three times at 11, 14, and 17 DAI (Days After Inoculation). During scoring, only the second and third leaves (Figure 3.6) were marked and assessed as only these two leaves developed and were infected at inoculation.

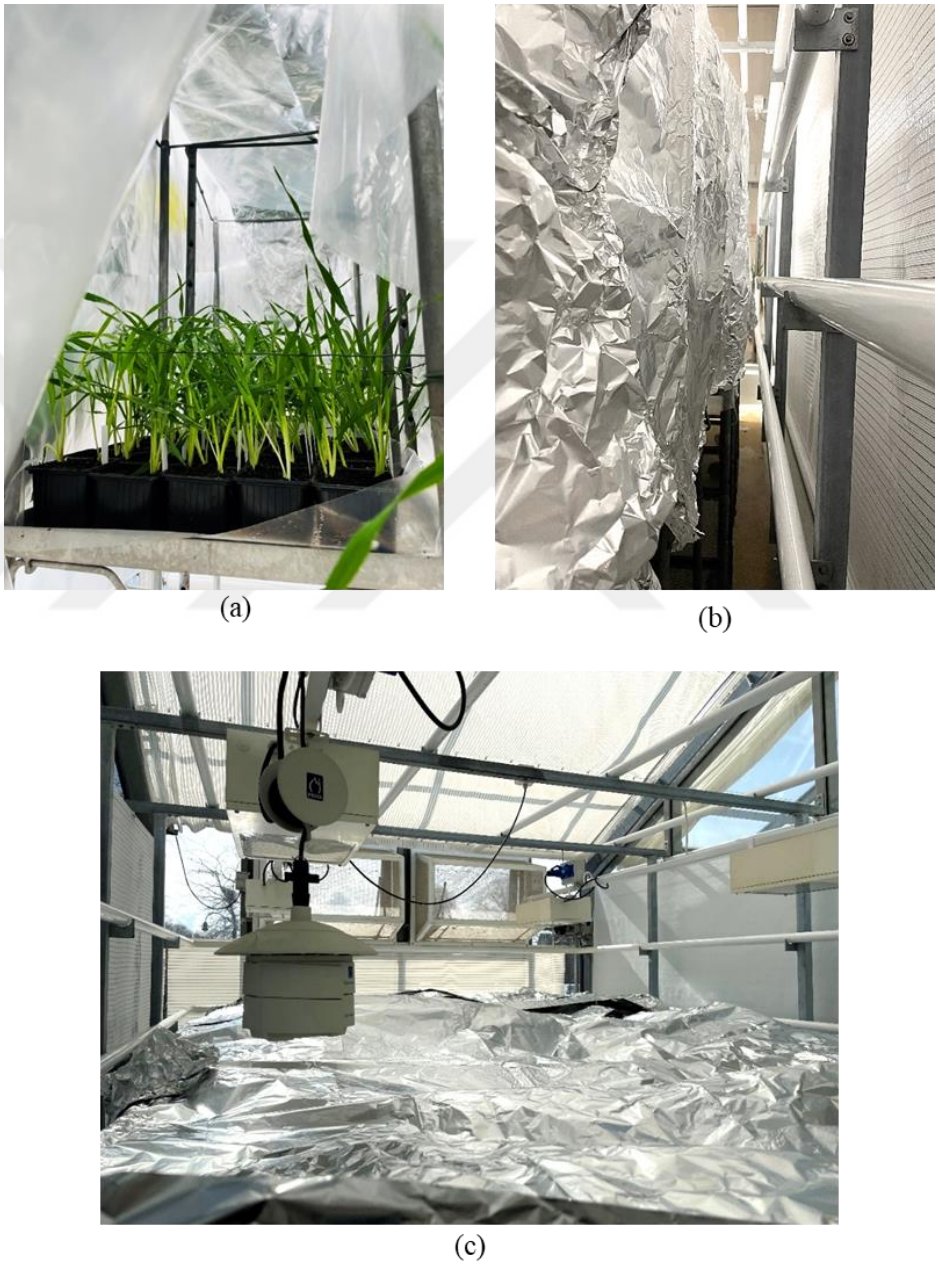

Figure 3.7. After-inoculation condition of the experiment (a) plants covered with plastic sheet immediately after inoculation (b) Side view of the chamber (c) Top view of the chamber. All trolleys were covered with plastic sheets as the first layer and then covered with Aluminum foil to prevent heating up because of respiration and evaporation.

Figure 3.8. Stages (1-10) of scald disease progressiveness in barley

After receiving the scoring results, the adjusted mean value for the tested barley genotypes in each scoring point (for individual replication) was calculated using the augmentedRCBD package in R statistical software using the following mathematical model:

$$Y_{il}=\mu+ G_{il}+ B_l + \varepsilon_{il}$$

where Y_{il} is the adjusted mean of i^{th} genotype in the l^{th} block; μ is the general mean value; G_{i1} is the effect of i^{th} genotype in the l^{th} block; B_1 is the block effect, and ε_{il} is the residual.

Based on the adjusted mean values obtained by the above calculation, the area under the disease progress curve (AUDPC) for individual replication was calculated. The AUDPC allows scholars to monitor the disease progression of the crop of interest through time (study period) (Shaner and Finney, 1976). Subsequently, the best linear unbiased prediction (BLUP) and broad-sense heritability (H^2) were calculated based on the resulting AUDPC values by using META-R 6.04 (Alvarado et al., 2015).

$$Y_{im} = \mu + G_{im} + R_m + \varepsilon_{im}$$

where Y_{im} is the BLUP of i^{th} genotype in m^{th} replicate, μ is the general mean value, G_{im} is the i^{th} genotype effect in m^{th} replicate, R_m is the effect of m^{th} replicate, and ε^{im} is the residual effect.

4. RESULTS

4.1 Disease Scoring

The seeds were sown on 11 March 2022, and inoculation was done on 28 March 2022. Scoring was started 11 days after inoculation and continued to second and third scorings with three-days interval until 14 April 2022. Initially, 292 barley genotypes were sown but depending on the plant survival during germination, only 284 genotypes remained and were scored together with four checks (Ingrid-C1, Freja-C2, RGT Planet-C3, and Laureate-C4) for both trial sets (augmented 1 and 2). The analysis of variance was done for two trial sets separately by the augmented RCBD package in RStudio.

4.2 Analysis of Variance (ANOVA)

The unadjusted and adjusted means of the treatment effect at all scoring times of augmented trial sets 1 and 2 showed a significant difference among the studied barley genotypes except for treatment adjusted mean scored at 14 DAI (days after inoculation). Table 4.1 and Table 4.2 show analysis of variance (ANOVA) results of the two augmented replicates, respectively. For the block effect, the unadjusted values of both trial sets showed a highly significant difference among the blocks. After adjusting the mean values with the checks, a significant difference among the blocks only occurred while scoring for the first time (11 DAI) in both trial sets. The descriptive and statistical explanations for the studied barley genotypes, including checks, were shown in Table 4.3 and Table 4.4 for the augmented replicates. The mean values during 11 DAI, 14 DAI, and 17 DAI for a replicate 1 were 4.41 ± 1.83, 8.21 ± 1.46, and 9.64 ± 1.18, respectively. Similarly, for the augmented trial set 2, the means were 4.59 ± 1.69 for 11 DAI, 9.15 ± 1.66 for 14 DAI, and 9.53 ± 1.31 for 17 DAI. Table 4.5 compares the mean scoring of the check cultivars and the overall adjusted means of all the tested barley genotypes at three scoring times for both trial sets. According to the table, for augmented set 1, RGT Planet and Laureate scored lower than the overall mean values of the first and second scoring time points; nevertheless, they surpassed the overall value at the final scoring. At the same time, the other checks, Ingrid and Freja, scored higher than the mean at all

scoring intervals. For augmented set 2, RGT Plante-C3 and Laureate-C4 had lower scores than the overall adjusted mean value for the first two scorings, but for the final scoring, only Laureate-C4 had a lower score than the overall adjusted mean value.

Table 4.1. Analysis of variance (ANOVA) of augmented block design for three scoring times in 284 genotypes and four checks of spring barley (Augmented trial set 1)

Source of variation	**Df***	**Scoring 1 (11 DAI)**	**Scoring 2 (14 DAI)**	**Scoring 3 (17 DAI)**
Treatment (adjusted)	287	3.42 **	2.11 ns	1.17 **
Treatment (unadjusted)	287	4.01 **	2.69 *	1.2 **
Block (adjusted)	13	3.53 *	2.65 ns	0.21 ns
Block (unadjusted)	13	16.54 **	15.46 **	0.81 *
Tested genotypes	283	3.78 **	2.65 *	1.21 **
Checks	3	25.7 **	6.09 *	0.07 ns
Checks vs. tested genotypes	1	2.69 ns	3.41 ns	0.78 ns
Residuals (error)	39	1.67	1.56	0.32

*Df = Degree of freedom

Table 4.2. Analysis of variance (ANOVA) of augmented block design for three scoring times in 284 genotypes and four checks of spring barley (Augmented trial set 2)

Source of variation	**Df***	**Scoring 1 (11 DAI)**	**Scoring 2 (14 DAI)**	**Scoring 3 (17 DAI)**
Treatment (adjusted)	287	2781 **	2.5 *	1.53 *
Treatment (unadjusted)	287	3.59 **	2.67 *	1.64 **
Block (adjusted)	13	2.81 **	0.82 ns	0.66 ns
Block (unadjusted)	13	20.91 **	4.51 **	3.12 **
Tested genotypes	283	3.43 **	2.66 *	1.66 **
Checks	3	18.51 **	3.86 ns	0.3 ns
Checks vs. tested genotypes	1	4.43 *	1.58 ns	0.03 ns
Residuals (error)	39	1.05	1.45	0.84

*Df = Degree of freedom

Table 4.3. Descriptive statistics of disease scorings at three-time points in 288 barley genotypes (Augmented trial set 1)

Trait	Count	Overall adj. mean	Std. Dev	Std. Error	Min	Max	CV
Scoring 1 (11 DAI)	288	4.41	1.83	0.11	0	9.29	29.58
Scoring 2 (14 DAI)	288	8.21	1.46	0.09	0.25	11	15.3
Scoring 3 (17 DAI)	288	9.64	1.18	0.07	1.08	10.39	5.85

Std. Dev= Standard deviation, Std. Error= Standard error, Min= Minimum, Max= Maximum, CV= Coefficient of variation

Table 4.4. Descriptive statistics of disease scorings at three-time points in 288 barley genotypes (Augmented trial set 2)

Trait	Count	Overall adj. mean	Std. Dev	Std. Error	Min	Max	CV
Scoring 1 (11 DAI)	288	4.59	1.69	0.1	0	8.6	22.57
Scoring 2 (14 DAI)	288	9.15	1.66	0.1	0.4	10.9	13.22
Scoring 3 (17 DAI)	288	9.53	1.31	0.08	0.62	11.12	9.64

Std. Dev= Standard deviation, Std. Error= Standard error, Min= Minimum, Max= Maximum, CV= Coefficient of variation

Table 4.5. Scoring of the check cultivars at three scoring points

Treatment	Augmented set 1			Augmented set 2		
	11 DAI	14 DAI	17 DAI	11 DAI	14 DAI	17 DAI
Freja	5.57	8.55	9.73	5.29	9.45	9.71
Ingrid	5.05	8.43	9.73	5.25	9.36	9.57
Laureate	2.79	7.21	9.88	3.07	8.38	9.36
RGT Planet	3.25	7.54	9.73	3.54	8.66	9.57
Overall adj. mean of all genotypes	4.41	8.21	9.64	4.59	9.15	9.53

4.3 Frequency Distribution

Figure 4.1 and Figure 4.2 showed the frequency distribution of the tested barley genotypes and four check cultivars in augmented trial sets 1 and 2, respectively. In the first time point scoring data showed that values were normally distributed (Figure 4.1 and Figure 4.2). However, as time progressed, values of the genotypes tended to be skewed in their distribution and were most skewed at the last time point of scoring (Figure 4.1 and Figure 4.2).

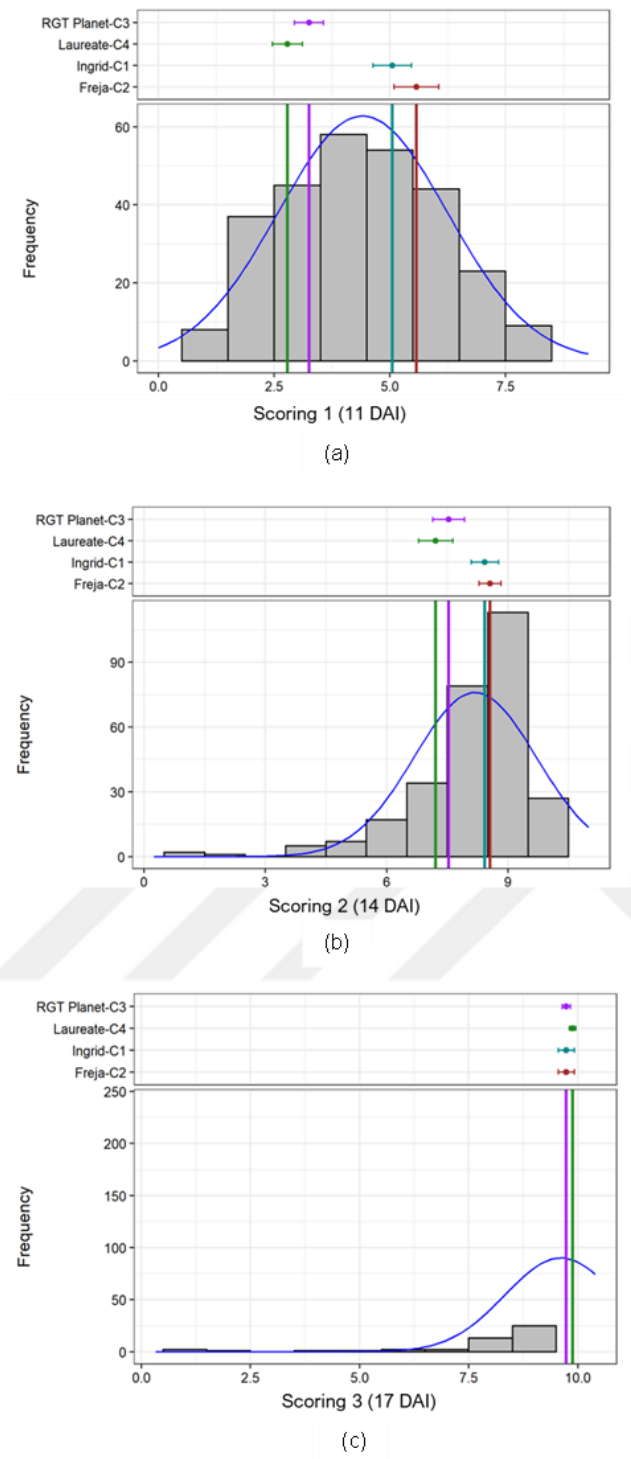

Figure 4.1. Frequency distribution of the tested barley genotypes in the augmented trial set 1 (a) first scoring at 11 DAI (b) second scoring at 14 DAI (c) third scoring at 17 DAI

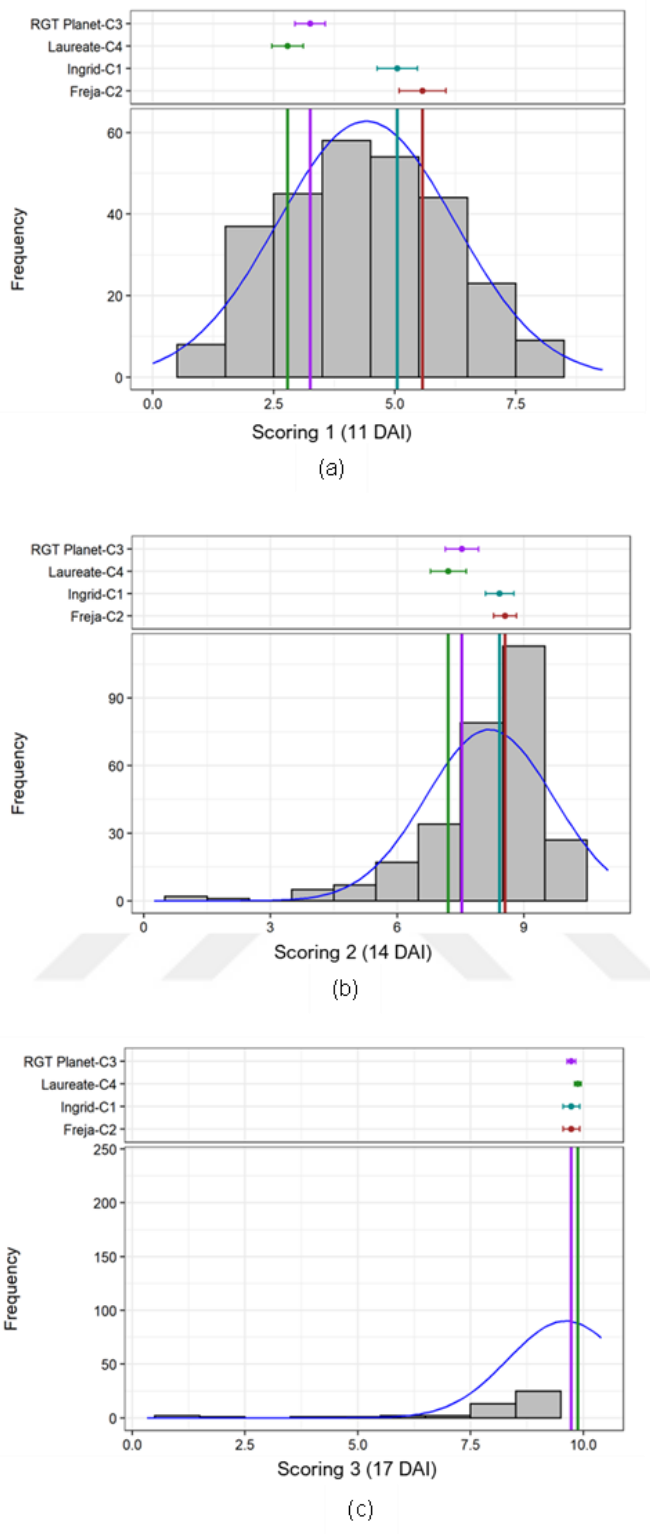

Figure 4.2. Frequency distribution of the tested barley genotypes in the augmented trial set 2 (a) first scoring at 11 DAI (b) second scoring at 14 DAI (c) third scoring at 17 DAI

4.4 The Area Under Disease Progress Curve (AUDPC)

The area under disease progress curve (AUDPC) is a valuable tool for monitoring disease progress over time (Shaner and Finney, 1976). In this experiment, the AUDPC values were calculated based on the adjusted means of the studied barley genotypes in each trial set. Figure 4.3 and Figure 4.4 illustrate the AUDPC of the barley genotypes and their distribution in terms of disease progression compared to the checks in both replicates.

For augmented set 1 (Figure 4.3), the AUDPC ranged from 3.09 to 56.43, where 59 genotypes equivalent to 20% of the total barley genotypes in the set 1 fell into the AUDPC range between 47.625 and 49.965. Additionally, nine barley genotypes reached the highest AUDPC vlaue (55.41-56.43), and on the contrary, three barley genotypes appeared to have the lowest AUDPC values within the range of 3.09 to 9.585.

On the other hand, for augmented set 2 (Figure 4.4), the barley genotypes attained an AUDPC range of 2.88 to 62.28. The influx of barley genotypes (89 genotypes) generally fell into an AUDPC range of 50.085 to 53.175. Four genotypes had the lowest AUDPC range (2.88 to 7.605), whereas two reached the highest AUDPC range between 60.375 and 62.28 in the augmented trial set 2.

Barley genotypes with the top five lowest AUDPC values were Lofa, Solar, ST-13947, Akka, and Rika in augmented set 1 and Lofa, Solar, Pongo, Akka, and ST-13947 in augmented set 2. Conversely, Bomi, Drost A, Vega, Pallas, and Freja (the one similar to one of the checks) were the top five ones possessing the highest AUDPC values in augmented set 1, and similarly, Herta, Foma, PR-9275, DS 9770-4, and DS 10060-4 in augmented set 2. The full list of AUDPC values for both augmented sets were mentioned in annex 1.

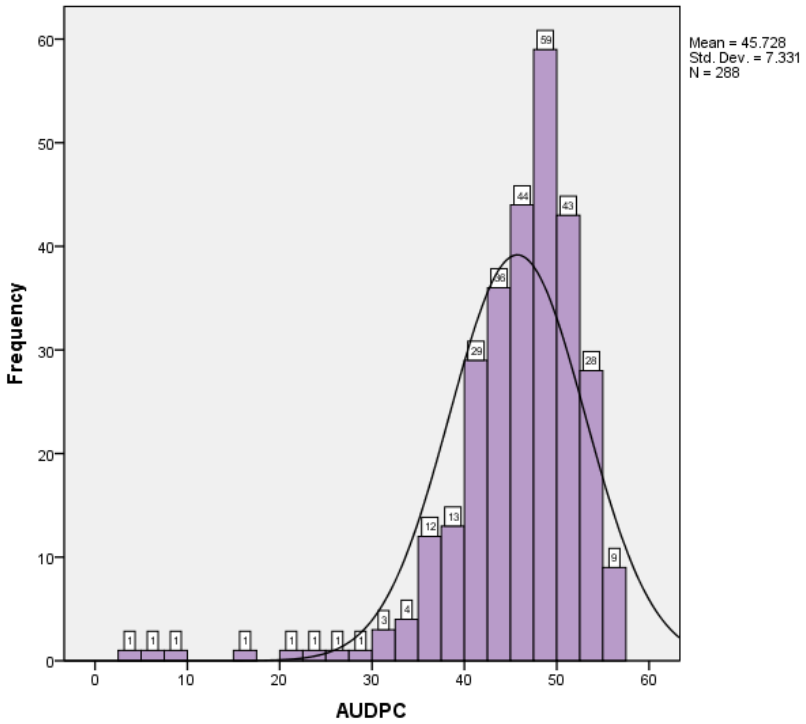

Figure 4.3. Area Under Disease Progress Curve (AUDPC) for the studied barley genotypes in augment set 1

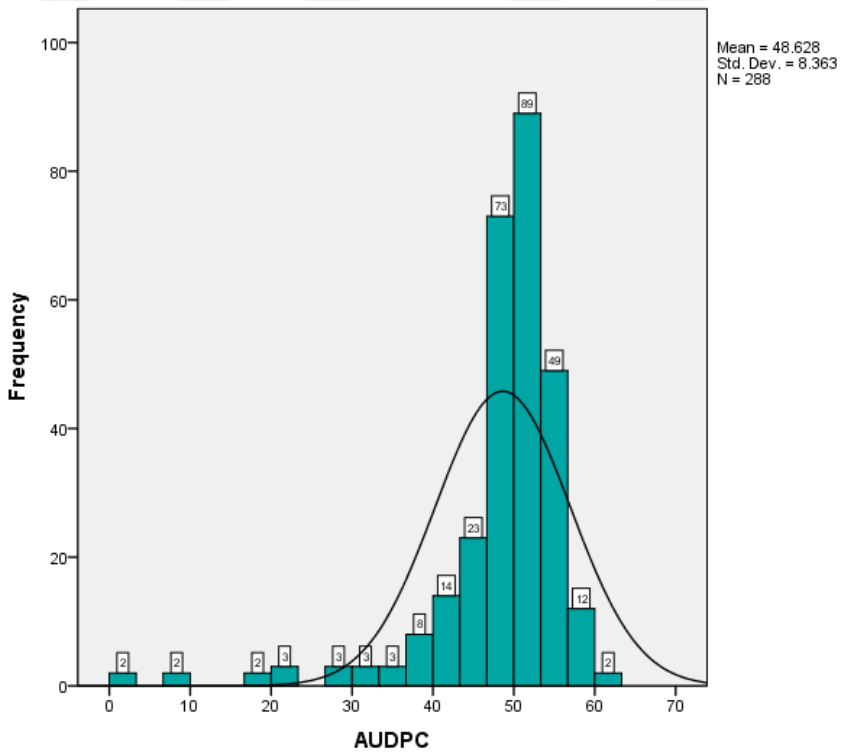

Figure 4.4. Area Under Disease Progress Curve (AUDPC) for the studied barley genotypes in augment set 2

4.5 Best Linear Unbiased Prediction (BLUP) of The Tested Barley Genotypes

For decades, plant breeders used the linear mixed model to obtain the basic linear unbiased prediction (BLUP) to predict the random effects and essential linear unbiased estimation (BLUE) to estimate the fixed effects. Plant breeders preferred to use BLUP between these two methods because BLUP values were more accurate than BLUE ones (Avrova and Knogge, 2012; Paulitz and Steffenson, 2011). In the current study, BLUEs for the studied barley genotypes were calculated based on the attained AUDPC values according to annex 1. Here, AUDPC values from both augmented trial sets, the BLUPs, BLUEs, and broad-sense heritability were analyzed using META-R software developed by CYMMIT. Table 4.6 shows a high heritability of 0.725099862 and the genotypic variance of 35.17599731. Furthermore, the grand mean of the genotypes was 47.17799479 with a coefficient of variation of 10.94680285. At the same time, Table 4.7 showed the complete list of studied barley genotypes and their BLUPs values in ascending order. According to the Table, Lofa, Solar, AKKA, St-13947, and Pongo were the genotypes possessing the lowest BLUP scores, while Herta, DS 9770-4, Meltan, Pallas, and Flavina attained the highest BLUP scores. Figure 4.5, 4.6 and 4.7 showed a comparative illustration of check genotypes, the high BLUP scored genotypes and low BLUP scored ones respectively.

Table 4.6. Heritability, genotype variance, grand mean and the coefficient of variation observed among the barley genotypes in the experiment

Statistic	BLUP-AUDPC	BLUE-AUDPC
Heritability	0.725099862	NA
Genotype Variance	35.17599731	NA
Residual Variance	26.67187514	26.67187526
Grand Mean	47.17799479	47.17799479
LSD	8.655836813	10.16506476
CV	10.94680285	10.94680287
n Replicates	2	2
Genotype significance	0	1.81608E-26

Table 4.7. Ranks of barley genotypes against scald disease according to the BLUP values

Genotypes	BLUP	Rank
LOFA	15.13366	1
Solar	15.94940	2
AKKA	21.49097	3
ST-13947	22.76896	4
Pongo	23.98169	5
Rika	28.06038	6
FRIDA	34.32524	7
5597.1.5.4	34.61891	8
Dziugiai	36.76158	9
DS 10058-4	37.57731	10
AKTA	37.74590	11
Diablo	39.75261	12
Olypus	39.99733	13
Ellinor	40.05716	14
LONE	40.39433	15
RG Mermeid	41.37321	16
OSTRIG	41.42216	17
5366.1.1	41.48741	18
Auksiniai	41.57443	19
SY Contour	41.69951	20
5501.7.2	41.75933	21
Hambo	41.92791	22
4882.1.5.3	41.96054	23
Maaren	42.10738	24
Cindy	42.18895	25
PUKE	42.20526	26
5525.1.1.3	42.30859	27
ERBIL	42.36841	28
ST-13965	42.91767	29
Ula	43.07538	30
Viking Gold	43.27660	31
KETI	43.29291	32
DROST	43.34730	33
DS 9879-6	43.41255	34
Laureate	43.57570	35
Acorn	43.62465	36
FERO	43.66271	37
Brioni	43.70622	38
HAVILA	43.72797	39
ROBERT	43.73341	40

Genotypes	BLUP	Rank
Leandra	43.76060	41
PR-7475.6	43.83674	42
PR-9234	44.05971	43
NFC Tipple	44.27180	44
DS 9898-4	44.30443	45
Accordine	44.54371	46
Lisen	44.61985	47
5635.2.2.1	44.64704	48
ST-13952	44.65248	49
Carmen	44.67967	50
RGT Planet	44.77756	51
5492.1.1.4	44.78843	52
FREJA	44.91895	53
BIRKA NGN2667	45.07122	54
DOMEN	45.10929	55
Tellus	45.14736	56
ST-13134	45.19630	57
Flair	45.22349	58
Lexy	45.23437	59
Ovation	45.23437	60
MAGNUM	45.28331	61
ST-13094	45.37032	62
Iskria	45.40295	63
SW Catriona	45.42471	64
VARUNDA	45.42471	65
4953.6.5.3.2	45.43015	66
Vilnie?iai	45.46277	67
TAARN	45.48453	68
MONA	45.49540	69
Elo	45.49540	70
NICKENDE BRAUGERSTE	45.49540	71
SWALLOW	45.59873	72
ST-13927	45.69118	73
SAMMY	45.72925	74
ST-13958	45.73469	75
WEISSE ERFURTER	45.89783	76
Aidas	45.92503	77
ST-13902	46.03923	78
SORT BYG	46.07730	79
Kirsna DS	46.08817	80

Genotypes	BLUP	Rank
KVL 210	46.11536	81
Cinnamon	46.11536	82
REX II	46.14799	83
ST-13876	46.15343	84
NYTSCHEVA 1104	46.27851	85
5467.1.2.5	46.30027	86
SW Cinnober	46.37640	87
Liisa	46.38184	88
DS 9873-6	46.38728	89
5534.1.4.3	46.44710	90
ABED 3171	46.47429	91
5365.2.2	46.50692	92
KVL 217	46.55042	93
DS 10009-4	46.55042	94
NAECKTE	46.58849	95
DS 9860-4	46.62112	96
Noja DS	46.64831	97
Anneli	46.65375	98
JENNY	46.65919	99
SCHWEIGERS ERIKA	46.65919	100
DS 10085-5	46.72445	101
Avalon	46.75164	102
PAMINA	46.82234	103
SUNE	46.82234	104
NUERNBERGER BYG	46.89303	105
KVL 212	46.92566	106
5681.7.8.2	46.95829	107
Highway	46.99636	108
ST-12890	47.12688	109
HERTA 5083	47.19758	110
Eifel	47.20301	111
HELLAS	47.21933	112
SALKA	47.30090	113
4533.4.3.6	47.33353	114
5226.9.4.1	47.37160	115
Axelina	47.43142	116
Aura DS	47.43142	117
SOLD	47.45861	118
4954.12.1.1.2	47.45861	119
RGT Astroid	47.46949	120

Genotypes	BLUP	Rank
NERY	47.50212	121
GRIT	47.52931	122
5591.1.9.4	47.55650	123
Jovita	47.63264	124
CARLSBERG II	47.70333	125
Vanja	47.71421	126
WELAM	47.74140	127
HARRY	47.74684	128
VISIR	47.76315	129
Paustian	47.78491	130
SENAT	47.87192	131
Dragoon	47.87192	132
5654.2.4.1	47.88280	133
EVA	47.90999	134
POLESSKIJ	47.90999	135
JO1072	47.91543	136
KVL 813	47.94262	137
Fender	47.96981	138
SW Barbra	48.00788	139
TYRA	48.03507	140
Honey	48.04594	141
ANLA	48.05682	142
JO0919	48.07857	143
Rusne DS	48.17102	144
DS 9440-9	48.24172	145
STRENGS FRANKEN III	48.25260	146
Margareta	48.27979	147
SALVE	48.29066	148
GUNNAR	48.31242	149
DINA	48.31786	150
Crescendo	48.38311	151
4638.6.2.7	48.38311	152
YMERBYG II	48.39399	153
INGRID	48.41574	154
ANNA	48.42118	155
Cosmopolitan	48.45381	156
PR-9250	48.45925	157
Elbo 6301	48.52451	158
4935.4.1.1	48.53539	159
Luoke	48.55170	160

Genotypes	BLUP	Rank
BREUNS WISA	48.61696	161
DS 10430-1	48.62240	162
5451.2.2	48.66047	163
ALIS	48.69309	164
Cecilia	48.69853	165
DCY69B	48.72572	166
ALVA	48.73116	167
SEJET 52/1494	48.75292	168
Propino	48.76379	169
HJA 77003	48.82361	170
Auksiniai II	48.82361	171
MOYJAR	48.82905	172
HJA 10076	48.85624	173
5486.1.2.1	48.87256	174
LINA	48.87800	175
ARAMIR	48.89431	176
LONG GLUMES	48.93238	177
SW Makof	48.93238	178
FLARE	48.93238	179
KVL 190	48.93782	180
DEBA	48.95957	181
NUTANS 187	48.96501	182
DS 9898-3	48.96501	183
FREJA	49.02483	184
ST-13899	49.03570	185
FREJA	49.07377	186
ALBERT	49.09553	187
KVL 367	49.14447	188
ZITA	49.23148	189
GORM	49.23148	190
RIMPAUS	49.23692	191
ST-13831	49.25867	192
BALDRIC	49.26955	193
STANGE	49.26955	194
4866.7.4.1.2	49.30218	195
AMSEL	49.30218	196
ST-12835	49.31306	197
ST-13955	49.41094	198
Arka DS	49.43813	199
CATRIN	49.50339	200

Genotypes	BLUP	Rank
KRISTINA	49.50339	201
ST-13893	49.50883	202
SPANIEN	49.57409	203
5436.7.4	49.57409	204
UFFE	49.57953	205
CAMTON	49.58497	206
Vilgott	49.60672	207
GUNHILD	49.67742	208
JONNA	49.67742	209
5656.1.3.2	49.70461	210
SEJET 51/1722	49.74268	211
Leeni	49.80794	212
SY Dolomit	49.81337	213
ROMI	49.84057	214
SEWA	49.87319	215
ST-13863	49.88407	216
5515.4.3	49.88407	217
SY Splender	49.92214	218
BIRGITTA	49.94389	219
HANKKIJAN AAPO	49.95477	220
RUPAL	50.03634	221
ST-13911	50.04722	222
DS 10367-6	50.08529	223
KVL 385	50.16686	224
MENTOR	50.18317	225
KWS Irina	50.23212	226
Fennica	50.25387	227
4668.1.2.1	50.25387	228
PR-9275	50.25931	229
ALF	50.25931	230
DS 10060-9	50.31913	231
DS 10261-14	50.31913	232
PERNILLA	50.35720	233
INGRID 4676	50.40070	234
ODIN	50.46053	235
GOLF	50.53122	236
ANSGAR	50.62911	237
NOLC. DRAEGER ALLERFR.	50.63455	238
SIMBA	50.63455	239
FREJA 5082	50.73244	240

Genotypes	BLUP	Rank
DOMEN 5089	50.75419	241
BALDER	50.76507	242
CRIMEE	50.76507	243
MIRJAM	50.77051	244
Mitja	50.79226	245
KVL 211	50.80314	246
NERY 1509	50.80314	247
GULL	50.83033	248
ST-13909	50.86296	249
DS 9857-3	50.90102	250
TAARN 1516	50.97172	251
DS 9879-5	50.99891	252
KARRI	51.00435	253
KARA	51.07505	254
D46-5-2-4-1	51.14575	255
HEINES HAHA	51.17294	256
ST-13963	51.17837	257
ST-13167	51.24363	258
IDA	51.24907	259
STALLAR I	51.30345	260
Ema DS	51.50467	261
Dotnuvos ketureiliai	51.51555	262
ST-12902	51.52098	263
RUPAL 1501	51.54818	264
NORDAL	51.62431	265
REFOMA	51.72764	266
ROLAND	51.84728	267
BIRKA NGN4712	51.85272	268
KENIA	51.89622	269
DROST A	51.99955	270
DS 10060-4	52.09200	271
ARLA	52.15726	272
Alsa	52.16270	273
TELLUS NGB	52.18989	274
BOMI	52.39654	275
KORU	52.53250	276
CAJA	52.60320	277
GALANT	52.60863	278
Trebon	52.97300	279
FOMA	52.99475	280

Genotypes	BLUP	Rank
KUSTAA	53.04369	281
VEGA	53.21228	282
4841.2.6.2	53.25579	283
FLAVINA	53.38087	284
PALLAS	53.86487	285
Meltan	53.93557	286
DS 9770-4	54.27818	287
HERTA	54.63710	288

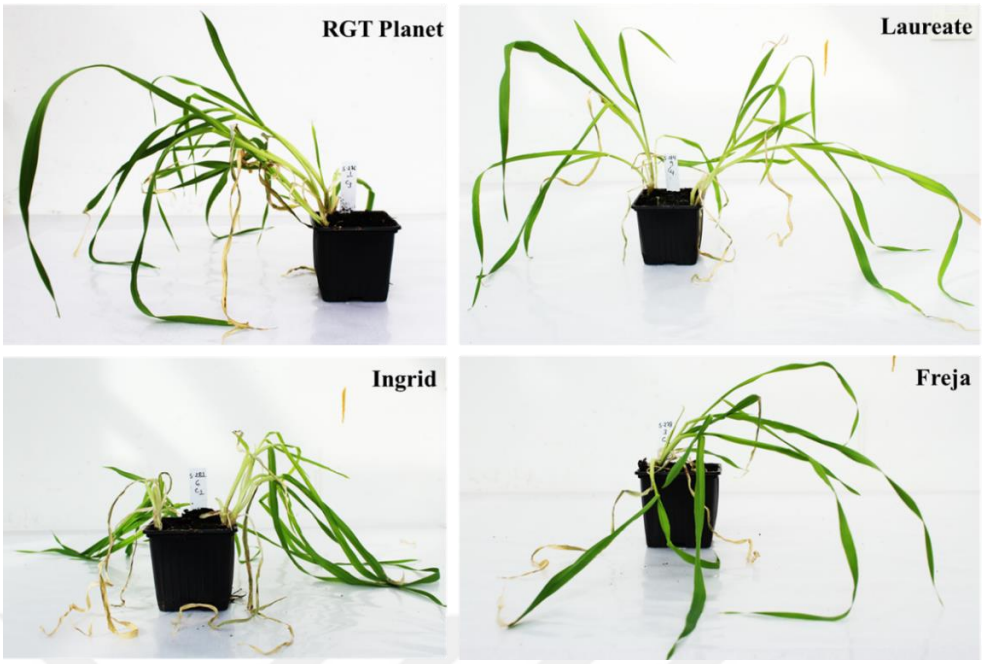

Figure 4.5. Status of check genotypes at 17 DAI

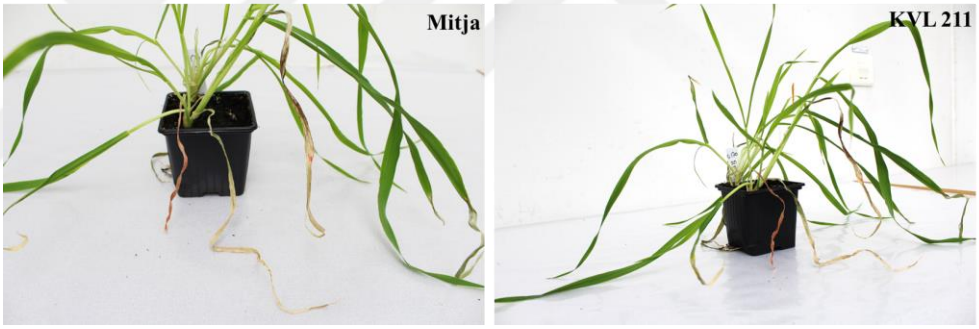

Figure 4.6. Status of barley genotypes having high AUDPC values (susceptible genotypes) at 17 DAI

Figure 4.7. Status of barley genotypes having low AUDPC values (resistant genotypes) at 17 DAI

5. DISCUSSION

This dissertation aimed to evaluate and identify the resistant barley genotypes against scald disease caused by the *Rhynchosporium commune.* The evaluation and identification of resistant genotypes were based on the phenotypic assessment (scoring) of the disease progression panel of a genebank barley germplasm genotypes in controlled environmental conditions.

Altogether with a prolonged existence of barley on the earth, its benefits are enormous to humans, though, at the same time, because of such extended presence, barley also faces different abiotic and biotic stresses through time. Among the biotic disturbance, the scald disease is one of the acute diseases in temperate regions. Under favorable climate conditions, scald disease can cause significant yield losses, especially in susceptible cultivars (Avrova and Knogge, 2012; Paulitz and Steffenson, 2011). Current standard practices to control scald disease are fungicide application, soil management such as tillage and crop rotation, and the use of resistant cultivars. Although resistance barley cultivars are already on the market, it is unpredictable how long the resistance will last, considering the evolutionary process of the pathogen over the plant defense system (e.g., mutation by deletion of NIP1 gene which is recognized by the Rrs1 gene in 3H chromosome (Rohe et al., 1995). Therefore, it is crucial for the breeders to develop barley cultivars with more persistent and robust resistance against scald disease. Consequently, to do so, resources such as germplasms including landraces and wild relatives would be the most appropriate genetic materials from which breeders would receive helpful information on barley cultivars with a diverse resistance. In order to use the valuable germplasms effectively, evaluating their performance under controlled environmental conditions such as greenhouse before field-level assessment would be vital in pre-breeding activities.

5.1 Culturing of *R. Commune* on CZV8CYM and Wheat Germ Agar (WGA) Media, Which Encouraged Mycelium Growth and Sporulation, Respectively

Although *R. commune*'s life cycle in the actual environment is not complicated by using the environmental resources such as soil and plant debris, specific growing conditions were necessary to obtain proper mycelium growth and sporulation for testing *R. commune* infection in plants. Even though different media were developed to culture *R. commune*, such as wheat germ agar, lima bean agar, CZV8CYM, and potato dextrose agar, the current protocol for using two types of media, CZV8CYM for mycelium growth and WGA for sporulation of *R. commune* in this experiment was optimized by the Department of Plant Breeding, SLU. By growing the fungi on WGA, the fungi produced relatively high amount of spore where the concentration of 1.35 x 10^6 conidia per ml of inoculation suspension was relatively possible to attain with low number of cultured plates. Inoculation was done by hand spraying the whole plant level (at the second and third leaves developing stage). Because of the invasion of fungal hyphae intracellularly, the epidermal collapse was observed at 8~10 DAI, indicating the hyphal growth inside plant cells. As a result, symptoms such as water-soaked lesions over the leaf surface were observed (Figure 5.1). Similar results of epidermal cell collapse and significant visualization of water-soaked lesions over the leaf surface were achieved by (Ayesu-Offei and Clare, 1970; Linsell et al., 2010; Lyngs Jørgensen et al., 1993).

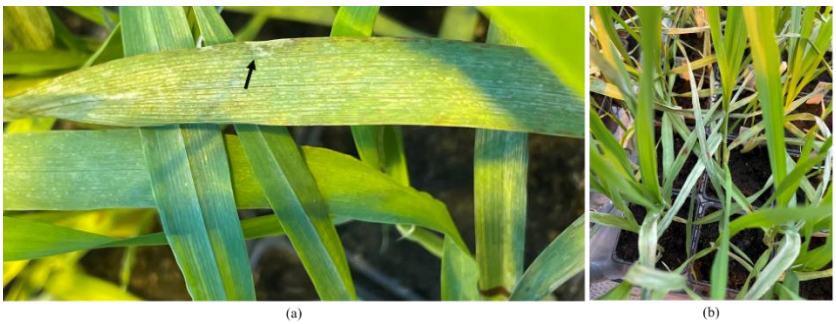

Figure 5.1. *R. commune* infected barley leaves (a) appearance of the water-soaked lesion at 9 DAI pointed with black arrow (b) droopy second and third leaves (marked ones) due to planting cell collapse resulting in a lack of leaf turgidity

5.2 Evaluation and Identification of Scald Resistant Barley Genotypes

The evaluation was performed from 11 DAI until 17 DAI, when at least 75% of the inoculated leaves reached the maximum infection score of 10. The collected scoring data were further analyzed by augmented RCBD package in RStudio, where the scoring results were adjusted by four check genotypes included in the experiment. The analysis of variance (ANOVA) represented that after adjusting the means scoring, a significant difference was observed among the tested barley genotypes in the augmented trial set 1 for all scoring times. Similarly, in the augmented trial set 2, a significant difference among the tested genotypes was presented except for the second scoring time (14 DAI). However, the block effect was not significant in both augmented sets. This result showed different responses of tested genotypes against scald throughout the whole infection period. However, no significant difference was observed when they were compared with the checks. This might be due to different resistance levels of the checks where RGT Planet and Laureate were known to be moderately resistant to scald while Ingrid and Freja were susceptible.

As a next step, the area under the disease progress curve (AUDPC) was calculated based on the adjusted mean values at specific scoring times. Two AUDPC curves were developed separately for augmented sets 1 and 2 (Figure 4.3 and Figure 4.4). The mean values were 45.728 (with a standard deviation of 7.331) and 48.628 (with a standard deviation of 8.363 for augmented set 1and for set 2, respectively. When two sets were jointly analyzed to find the best linear unbiased prediction (BLUP), the average AUDPC was 47.178. The top five genotypes with the lowest AUDPC were Lofa, Solar, ST-13947, Akka, and Rika for set 1 and Lofa, Solar, Pongo, Akka, and ST-13947 for set 2. After a combined analysis of two trial sets, five genotypes with the lowest AUDPC were Lofa, Solar, Akka, St-13947, and Pongo. Out of five genotypes with the lowest AUDPC values, four genotypes were identical to the finding of a separate analysis of each trial set. Based on this phenotypic information, it could be assumed that these four genotypes had a stable resistant level against scald. On the other hand, there were no common genotypes between the two trial sets when reviewing the top five genotypes with the highest AUDPC. However, the combined analysis of two trial sets revealed that Herta, DS-

9770-4, Meltan, Pallas, and Flavina were the top five genotypes with the highest AUDPC values.

However, when comparing these results with the ones from combined analysis (BLUPs) separately, two genotypes from each trial set were found in the combined analysis. Those genotypes were Pallas, and Malta from trial set 1 and Herta and DS-9770-4 from trial set 2. To confirm these results, further experiments under field conditions should be carried out to reveal their resistance against scald. disease. In terms of heritability, a considerable estimate of 0.725 was obtained while comparing with the other experiments conducted in either field or controlled conditions by (Aoki et al., 2011; Spanner et al., 1998; Xi et al., 2019) which got a broad-sensed heritability estimate within a range of 0.20-0.97. This high heritability estimate revealed that variation (resistance and susceptibility) among the barley genotypes was mainly due to the genotypic effect. Hence, the genotypes with the lowest BLUP values could be considered for future breeding programs in developing scald resistance barley genotypes.

6. CONCLUSION AND FUTURE DIRECTIONS

This study allowed the understanding of how *Rhynchosporium commune* infection progressed with time and the response of each barley genotype against this disease. Although different responses were observed among the tested 284 genotypes, eventually, five genotypes could be resistant genotypes, potentially retaining high quantitative resistance to scald disease.

In conducting experiments related to resistance breeding, every step of the inoculation process, including pathogen culturing, inoculum preparation, and inoculation itself, is crucial to strictly follow the protocols and guidelines for proper and effective inoculation. Additionally, after inoculation in this experiment, the climatized chamber was supplied with a constant temperature and a humidity of 17 °C and 80%, respectively, throughout the experimental period so that an ultimate scald infection occurred in all inoculated genotypes.

The Best Linear Unbiased Predicts (BLUP) based on the area under disease progress curve (AUDPC) values revealed five genotypes ranked at the top with the lowest AUDPC values, which meant the most resistant barley genotypes among the tested barley ones. These genotypes were Lofa, Solar, Akka, St-13947, and Pongo. (Qualset, 1975) stated that exploitation of germplasms such as wild ancestors and landraces maintained in the gene banks is the key to the success of resistance breeding. Moreover, a high broad-sensed heritability (H^2) estimate of 0.72 showed that the resistance found in these genotypes was mainly due to their genetic factor. To confirm these results, field trials under actual field conditions should be carried out, and the performance of these superior genotypes should be evaluated in a multi-location environment trials. The other approach would be incorporating marker-assisted selection (MAS) to identify scald resistance QTLs in these genotypes and speed up the selection process for scald resistance barley genotypes.

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ANNEXS

Annex 1. The area under disease progress curve (AUDPC) values of the tested barley genotypes

Annex 1. The area under disease progress curve (AUDPC) values of the tested barley genotypes

Genotype	AUDPC	Rank
LOFA	3.09	1
Solar	5.34	2
ST-13947	9.585	3
AKKA	15.9	4
Rika	22.035	5
Pongo	22.785	6
LONE	26.04	7
OSTRIG	29.445	8
Diablo	31.56	9
DROST	32.025	10
ST-13134	32.235	11
Dziugiai	33.075	12
DS 10058-4	33.555	13
Hambo	33.75	14
Acorn	34.95	15
PR-9234	35.04	16
Leandra	35.34	17
SW Catriona	35.61	18
ROBERT	35.91	19
FERO	36.09	20
Lisen	36.18	21
Anneli	36.36	22
BIRKA NGN2667	36.405	23
FREJA	36.465	24
ST-13952	36.465	25
KETI	36.555	26
ST-13965	36.555	27
5597.1.5.4	38.055	28
5366.1.1	38.34	29
Olypus	38.34	30
Ula	38.4	31
SORT BYG	38.7	32
FRIDA	38.715	33
VARUNDA	38.805	34
NFC Tipple	38.805	35
RG Mermeid	39.435	36
AKTA	39.465	37
Flair	39.735	38
ANLA	39.75	39
SY Contour	39.84	40

Genotype	AUDPC	Rank
Carmen	40.125	41
Maaren	40.215	42
Tellus	40.29	43
4882.1.5.3	40.29	44
DS 9860-4	40.5	45
MONA	40.5	46
MAGNUM	40.56	47
Laureate	40.635	48
WEISSE ERFURTER	40.665	49
RGT Astroid	40.785	50
Margareta	40.86	51
ST-13927	40.935	52
HAVILA	40.95	53
ST-13876	40.965	54
Ovation	40.965	55
Kirsna DS	41.16	56
Aura DS	41.235	57
5226.9.4.1	41.235	58
TAARN	41.31	59
Viking Gold	41.4	60
5635.2.2.1	41.43	61
REX II	41.715	62
SUNE	42.09	63
KVL 217	42.09	64
RGT Planet	42.09	65
ALVA	42.36	66
Liisa	42.375	67
5525.1.1.3	42.375	68
Propino	42.45	69
5654.2.4.1	42.555	70
PR-7475.6	42.555	71
Jovita	42.735	72
GRIT	42.735	73
Cinnamon	42.84	74
Luoke	42.9	75
ST-13958	43.125	76
DOMEN	43.185	77
DS 9873-6	43.215	78
Iskria	43.215	79
SAMMY	43.29	80

Genotype	AUDPC	Rank
4533.4.3.6	43.305	81
5534.1.4.3	43.5	82
GORM	43.575	83
Axelina	43.65	84
SENAT	43.665	85
HERTA 5083	43.68	86
SALVE	43.86	87
Elo	43.875	88
ABED 3171	43.935	89
Auksiniai	43.935	90
PR-9275	43.95	91
5486.1.2.1	43.965	92
4954.12.1.1.2	44.04	93
PR-9250	44.055	94
NYTSCHEVA 1104	44.055	95
ST-13902	44.055	96
AMSEL	44.31	97
KVL 212	44.325	98
HARRY	44.535	99
DINA	44.61	100
STRENGS FRANKEN III	44.7	101
5492.1.1.4	44.7	102
Cecilia	44.715	103
CARLSBERG II	44.715	104
SCHWEIGERS ERIKA	44.715	105
Fender	45.06	106
CAMTON	45.09	107
DS 10085-5	45.09	108
NAECKTE	45.09	109
5467.1.2.5	45.15	110
DS 10009-4	45.165	111
ST-12890	45.18	112
ANNA	45.36	113
SW Cinnober	45.36	114
Crescendo	45.435	115
5365.2.2	45.435	116
PAMINA	45.45	117
DS 9879-6	45.525	118
4953.6.5.3.2	45.54	119
Eifel	45.555	120

Genotype	AUDPC	Rank
Paustian	45.66	121
DCY69B	45.735	122
TYRA	45.81	123
MOYJAR	45.825	124
LONG GLUMES	45.84	125
NUERNBERGER BYG	45.84	126
DS 9898-4	45.84	127
HELLAS	45.9	128
SOLD	45.915	129
ST-13094	45.93	130
WELAM	46.215	131
Vanja	46.215	132
Highway	46.305	133
BALDRIC	46.485	134
INGRID 4676	46.59	135
DS 10430-1	46.68	136
Avalon	46.68	137
Elbo 6301	46.95	138
ST-13899	46.965	139
NICKENDE BRAUGERSTE	46.965	140
Brioni	46.965	141
HJA 10076	47.04	142
Dragoon	47.055	143
Vilnie?iai	47.055	144
Accordine	47.055	145
5591.1.9.4	47.31	146
DS 9857-3	47.34	147
4638.6.2.7	47.415	148
INGRID	47.46	149
ALIS	47.625	150
SW Barbra	47.685	151
Leeni	47.7	152
NUTANS 187	47.715	153
JENNY	47.715	154
5501.7.2	47.715	155
Aidas	47.775	156
ODIN	47.79	157
UFFE	47.805	158
SALKA	47.805	159
STANGE	47.91	160

Genotype	AUDPC	Rank
Rusne DS	48.06	161
KVL 190	48.09	162
LINA	48.105	163
ST-13911	48.15	164
ST-13831	48.15	165
FREJA 5082	48.165	166
5436.7.4	48.165	167
KVL 210	48.18	168
ERBIL	48.18	169
ST-13893	48.285	170
5656.1.3.2	48.36	171
YMERBYG II	48.375	172
4668.1.2.1	48.465	173
Cosmopolitan	48.465	174
POLESSKIJ	48.465	175
Ema DS	48.54	176
SEJET 52/1494	48.54	177
DS 10367-6	48.555	178
KVL 813	48.555	179
FREJA	48.6	180
KARA	48.75	181
SEJET 51/1722	48.81	182
ALBERT	48.825	183
NERY 1509	48.84	184
GOLF	48.84	185
HANKKIJAN AAPO	48.84	186
ST-13863	48.84	187
5681.7.8.2	48.84	188
KVL 211	48.93	189
JONNA	48.93	190
SPANIEN	49.125	191
VISIR	49.185	192
5451.2.2	49.215	193
JO1072	49.215	194
DS 10060-4	49.41	195
FLARE	49.5	196
ANSGAR	49.575	197
SEWA	49.575	198
GULL	49.59	199
SY Splender	49.59	200

Genotype	AUDPC	Rank
RIMPAUS	49.59	201
Lexy	49.59	202
IDA	49.68	203
SW Makof	49.68	204
ROMI	49.86	205
4866.7.4.1.2	49.875	206
KVL 367	49.875	207
NOLC. DRAEGER ALLERFR.	49.965	208
FOMA	50.025	209
ZITA	50.055	210
Auksiniai II	50.055	211
Noja DS	50.055	212
ARAMIR	50.235	213
Vilgott	50.34	214
TELLUS NGB	50.43	215
ST-13955	50.43	216
HJA 77003	50.43	217
Honey	50.43	218
Dotnuvos ketureiliai	50.535	219
BIRGITTA	50.7	220
TAARN 1516	50.715	221
KWS Irina	50.715	222
SWALLOW	50.715	223
Fennica	50.805	224
SIMBA	50.91	225
5515.4.3	50.91	226
DEBA	50.985	227
Arka DS	51	228
CRIMEE	51.075	229
Cindy	51.15	230
GUNNAR	51.165	231
ST-13909	51.435	232
BREUNS WISA	51.45	233
BIRKA NGN4712	51.465	234
ST-12902	51.465	235
PERNILLA	51.465	236
ALF	51.465	237
DS 9898-3	51.465	238
JO0919	51.525	239
Ellinor	51.525	240

Genotype	AUDPC	Rank
CATRIN	51.555	241
REFOMA	51.84	242
SY Dolomit	51.84	243
DS 9440-9	51.9	244
DS 10060-9	51.93	245
4935.4.1.1	52.035	246
ROLAND	52.185	247
ST-13963	52.215	248
STALLAR I	52.275	249
BALDER	52.305	250
PUKE	52.41	251
DS 10261-14	52.575	252
Trebon	52.59	253
ARLA	52.59	254
D46-5-2-4-1	52.59	255
KARRI	52.59	256
GUNHILD	52.59	257
HERTA	52.65	258
GALANT	52.68	259
KENIA	52.68	260
DS 9879-5	52.68	261
ST-12835	52.68	262
EVA	52.68	263
KVL 385	52.785	264
DOMEN 5089	52.935	265
ST-13167	52.965	266
HEINES HAHA	53.055	267
NERY	53.055	268
4841.2.6.2	53.34	269
Mitja	53.4	270
MIRJAM	53.43	271
KORU	53.91	272
KRISTINA	54.18	273
FLAVINA	54.465	274
MENTOR	54.555	275
KUSTAA	54.66	276
NORDAL	54.66	277
RUPAL	54.9	278
CAJA	54.93	279
DS 9770-4	55.41	280

Genotype	AUDPC	Rank
Alsa	55.5	281
Meltan	55.59	282
RUPAL 1501	55.59	283
FREJA	55.59	284
PALLAS	56.055	285
VEGA	56.055	286
DROST A	56.34	287
BOMI	56.43	288