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The Use of Algorithms and Molecular Markers to Identify Sources of Scald Resistance (Rhynchosporium commune) in Different Subsets of Hordeum Genetic Resources

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Preamble

The work presented in this thesis was carried out at the International Center of Agricultural Research in the Dry Areas (ICARDA) in collaboration with the Laboratory of Microbiology and Molecular Biology, Faculty of Sciences, BioBio Research Center, University Mohammed V in Rabat, Morocco, under the supervision of Professor **Belkadi Bouchra, Pr. Abdelkarim Filali-Maltouf,** and **Dr. Amri Ahmed**.

This study aimed at providing new sources of resistance to scald in barley along with the associated markers which will be highly valuable for barley breeding efforts in Morocco and globally.

It has covered different aspects or research spanning along the continuum of conservation and use of genetic resources using different mining approaches of genetic resources, identification of novel sources of resistance at seedling and adult plant stages, and identification of genomic regions associate with resistance and using machine learning for the selection of best-bet subsets for resistance to scald disease from the large number of genebank accessions.

It has also allowed to adopt a reliable approach for isolation and screening of germplasm to scald.

Dedication

This thesis is dedicated to those who have supported and guided me throughout this journey.

My beloved family, whose unconditional love, support, and patience have been my foundation throughout this journey.

To my parents, for their sacrifices, encouragement, and constant belief in my dreams. Your strength and guidance have always inspired me to strive for excellence.

To my mentors, for your wisdom, guidance, and faith in my abilities. You have been an inspiration and a source of knowledge that I will always cherish.

To my friends and colleagues, for your companionship and shared experiences. Thank you for being a part of this challenging yet fulfilling path. And to the memory of those who are no longer with us, but whose influence and love continue to resonate within me.

Finally, to the pursuit of knowledge and discovery, may this work contribute a small piece to the vast puzzle of understanding the world around us.

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Résumé

L'orge (*Hordeum vulgare L*.) est l'une des premières cultures domestiquées, reconnue pour son adaptation aux environnements difficiles et ses multiples usages, la production et la qualité de l'orge sont affectées par plusieurs phytopathogènes, y compris *Rhynchosporium commune*. Cette recherche visait à identifier des sources de résistance au scald dans divers sous-ensembles de ressources génétiques et de marqueurs moléculaires associés en utilisant des études d'association pangénomiques. Les sous-ensembles GCP, FIGS et une collection de l'orge sauvage reçus de la banque de gènes et un panel construit par les sélectionneurs (AM17). L'évaluation de la résistance a été réalisée au stade plantule (SRT) dans des conditions contrôlées et au stade plante adulte (APR) à Marchouch, Rommani et Guich au Maroc et à Holetta en Éthiopie. Tous les sous-ensembles ont révélé des sources de résistance à la rhynchosporiose aux stades SRT et APR, mais avec des pourcentages différents entre les isolats et entre les sites. FIGS a montré des pourcentages relativement plus élevés d'accessions résistantes par rapport à GCP, démontrant l'efficacité de l'approche FIGS. Quatre modèles d'apprentissage automatique ont été ajustés sur des ensembles d'entraînement pour prédire les réactions au scald sur les ensembles de test basés sur diverses métriques. Tous les modèles ont efficacement identifié les accessions résistantes avec des spécificités supérieures à 0.88 mais ont montré des performances différentes. Les études d'association pangénomiques (GWAS) réalisées en utilisant la puce SNP Illumina iSelect 50K ont permis d'identifier des régions génomiques associées à la résistance au scald. Pour le panel AM17 caractérisé avec 36 793 SNPs polymorphes, 102 MTA (15 QTL) au stade SRT et 25 MTA (12 QTL) au stade APR sont associés à la résistance à la maladie et sont prédominant dans les chromosomes 3H et 7H, avec 9 QTLs considérés nouveaux. Pour *H. spontaneum*, les 26253 marqueurs SNP identifiés ont permis de subdiviser les accessions en trois sous-populations basées sur l'origine géographique, avec la sous-population d'Asie de l'Ouest ayant le nombre le plus élevé d'allèles privés et de richesse allélique. GWAS a permis d'identifier 24 MTA au stade SRT et 31 MTA au stade APR qui sont reparties principalement sur les chromosomes 2H, 3H et 7H, avec 27 MTA considérés nouveaux. Les séquences des marqueurs SNP significatifs aux deux stades se trouvent dans

des régions génomiques enrichies en protéines fonctionnelles impliquées dans divers processus cellulaires, y compris la résistance aux maladies.

Mots-clés : *Hordeum* spp, ressources génétiques, *Rhynchosporium commune*, algorithmes d'apprentissage automatique, génome, gènes candidats, marqueurs SNP.

Abstract

Barley (*Hordeum vulgare L*) is one of the first cultivated crop, known for its adaptation to harsh environments and have multiple uses as food, feed, and malt. However, barley production and quality are affected by several phytopathogens including *Rhynchosporium commune*. This research aimed to identify sources of resistance to scald in various subsets of barley germplasm and of the associated molecular markers using (GWAS). The subsets GCP, FIGS, accessions of *Hordeum spontaneum* from ICARDA genebank, and a breeder panel (AM17) were screened for resistance at seedling (SRT) in greenhouse and adult plant stage (APR) at Marchouch, Rommani and Guich sites in Morocco and at Holetta-Ethiopia. All subsets revealed sources of resistance to scald at both SRT and APR, but with different percentages between isolates and among sites. FIGS showed relatively higher percents of resistant accessions at SRT and APR compared to GCP showing the effectiveness of the FIGS approach. Four machine learning models were tuned on training sets to predict scald reactions on the test sets based on diverse metrics. All models efficiently identified resistant accessions with specificities higher than 0.88 but showed different performances between isolates at the seedling and to field populations at the adult plant stage. The Genome wide association studies (GWAS) using Illumina iSelect 50K SNP array allowed to identify 102 MTA (15 QTL) at SRT, and 25 MTA (12 QTL) at APR associated with scald resistance in AM17, mostly in chromosomes 3H and 7H with 9 novel QTLs. For *H. spontaneum* subset, the 26,253 identified SNP markers allowed the accessions to be subdivided into three sub-populations based on geographic origin, with the West Asian sub-population having the highest number of private alleles and allelic richness. GWAS allowed to detect 24 MTA at SRT and 31 MTA at APR were associated with scald resistance with 27 MTA identified as novel. The sequences of the significant SNP identified were located in genomic regions enriched with functional proteins involved in diverse cellular processes including disease resistance. The identified sources of resistance and associated molecular markers can be used to develop durable resistance to scald by barely breeding programs.

Key words*:* *Hordeum* spp, genetic resources, *Rhynchosporium commune*, machine learning, genome, candidate genes, SNP markers.

Résumé détaillé

L'orge (*Hordeum vulgare L*.) est l'une des premières cultures domestiquées, reconnue pour son adaptation aux environnements difficiles et ses multiples usages comme aliment, fourrage, et malt. Cependant, la production et la qualité de l'orge sont affectées par plusieurs phytopathogènes, y compris la rhynchosporiose, causée par le pathogène hémibiotrophe *Rhynchosporium commune*. Le développement de variétés hautement productives et résistantes est clé pour surmonter ce défi. Cette recherche visait à identifier des sources de résistance à la rhynchosporiose dans divers sous-ensembles de ressources génétiques et de marqueurs moléculaires associés en utilisant des études d'association pangénomiques. Les sous-ensembles GCP (Génération challenge program), FIGS (Focused Identification of Germplasm Strategy) et une collection de l'orge sauvage (*Hordeum spontaneum*) reçus de la banque de gènes et un panel construit par les sélectionneurs (AM17). L'évaluation de la résistance a été réalisée au stade plantule (SRT) dans des conditions contrôlées et au stade plante adulte (APR) à Marchouch, Rommani et Guich au Maroc pour tous les ensembles et à Holetta-Éthiopie pour FIGS et GCP. Tous les sous-ensembles ont révélé des sources de résistance à la rhynchosporiose aux stades SRT et APR, mais avec des pourcentages différents entre les isolats et entre les sites. FIGS a montré des pourcentages relativement plus élevés d'accessions résistantes au SRT et à l'APR par rapport à GCP, démontrant l'efficacité de l'approche FIGS. Quatre modèles d'apprentissage automatique ont été ajustés sur des ensembles d'entraînement pour prédire les réactions au scald sur les ensembles de test basés sur diverses métriques. Tous les modèles ont efficacement identifié les accessions résistantes avec des spécificités supérieures à 0.88 mais ont montré des performances différentes. L'évaluation de 316 entrées du panel AM17 a montré qu'au stade SRT 19,9% et 30,3% pour l'isolat SC-S6, 6,7% et 23,3% pour SC-511, et 6,5% et 5% pour SC-1122 étaient respectivement immunes et résistantes avec 11 génotypes résistants aux trois isolats. Au APR, 29% étaient résistants à Marchouch, 65% à Rommani et 55% à Guich avec 56 accessions résistantes dans les trois sites. L'évaluation de 103 accessions d'*Hordeum spontaneum* a montré 18% sont immunes et 27% résistantes pour l'isolat SC-511 tandis que seulement 34% des accessions sont résistantes à SC-1122, avec dix accessions résistantes aux deux isolats. A APR, 30% et 26% des accessions étaient respectivement résistantes à Marchouch et Guich avec seulement cinq accessions résistantes dans les deux sites. Les études d'association pangénomiques (GWAS) réalisées en utilisant la puce SNP Illumina iSelect 50K ont permis d'identifier des régions génomiques associées à la résistance à la rhynchosporiose aux stades SRT et APR. Pour le panel AM17 caractérisé avec 36793 SNPs polymorphes, 102 MTA (15 QTL) au stade SRT et 25 MTA (12 QTL) au stade APR sont associés à la résistance à la maladie et sont prédominant dans les chromosomes 3H et 7H, avec 9 QTLs considérés nouveaux. Pour *H. spontaneum*, les 26253 marqueurs SNP identifiés ont permis de subdiviser les accessions en trois sous-populations basées sur l'origine géographique, avec la sous-population d'Asie de l'Ouest ayant le nombre le plus élevé d'allèles privés) et de richesse allélique. GWAS a permis d'identifier 24 MTAs au stade SRT et 31 MTAs au stade APR associés à la résistance à la rhynchosporiose, et sont reparties principalement sur les chromosomes 2H, 3H et 7H. Les séquences des marqueurs SNP significatifs aux deux stades se trouvent dans des régions génomiques enrichies en protéines fonctionnelles impliquées dans divers processus cellulaires, y compris la résistance aux maladies. Les sources de résistance identifiées et les marqueurs moléculaires associés seront utilisés dans une stratégie

de déploiement de gènes pour assurer une protection durable de l'orge contre la rhynchosporiose.

Mots-clés : *Hordeum* spp, ressources génétiques, *Rhynchosporium commune*, algorithmes d'apprentissage automatique, génome, gènes candidats, marqueurs SNP.

List of publications

Houda Hiddar, Sajid Rehman, Berhane Lakew, Ramesh Verma, MuamarAl-Jaboobi, Adil Moulakat, Zakaria Kehel, Abdelkarim Filali-Maltouf, Michael Baum, Ahmed Amri (2021). **Assessment and modeling using machine learning of resistance to scald (*Rhynchosporium commune*) in two specific barley genetic resources subsets. Nature Scientific Reports**, https://doi.org/10.1038/s41598-021-94587-6

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Nabil Meskini, Bilal Aghoutane, **Houda Hiddar**, Tanvir Islam, Mohammed El Ghzaoui, Hanan El Faylali (2023). **Modeling a Square Slotted Antenna for 5G Applications using an Equivalent Circuit Approach. Journal of Nano- and Electronic Physics**, Volume 15, Number 4, https://doi.org/10.21272/jnep.15(4).04030

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H. Hiddar, B. Belkadi, S. Rehman, B. Lakew, R. Verma, M. Al-Jaboobi, A. Moulakat, Z. Kehel, A. Filali-Maltouf, M. Baum, A. Amri (2022) "**Assessment and modeling using machine learning of resistance to scald (*Rhynchosporium commune*) in two specific barley genetic resources subsets**". At the International Symposium on Cereal Leaf Blights, Gammarth, Tunisia, May 11-12-13, 2022. **Oral**

H. Hiddar, S. Rehman, A. Filali Maltouf, Z. Kehel, A. Amri (2018) "**Evaluation of FIGS set for resistance to scald disease (*Rhynchosporium commune*)**". Afro-Med conference, Rabat, Morocco, July 7-8. **Oral**

H. Hiddar, S. Rehman., A. Amri, A. Filali Maltouf, M. Baum, Z. Kehel (2017) "**Screening of Moroccan varieties set for resistance to scald disease (*Rhynchosporium commune*)**". At the AMPP Symposium, ENA Meknes, November 14. **Oral**

H. Hiddar, A. Amri, A. Filali Maltouf, S. Rehman., Z. Kehel, M. Baum (2017) "**Evaluation of divers sets of barley germplasm for resistance to scald disease (*Rhynchosporium commune*)**". At the Second International Workshop on Barley Leaf Diseases, ICARDA, Rabat, Morocco, April 5-7, 2017 **Poster**

H. Hiddar, A. Amri, A. Filali-Maltouf, S. Rehman, Z. Kehel, M. Baum (2017) "**Evaluation of diverse sets of barley germplasm for resistance to scald disease (*Rhynchosporium commune*)**". At the First International Student Symposium, ICARDA, Rabat, Morocco, January 20, 2017. **Oral**

H. Hiddar, A. Amri, A. Filali-Maltouf, M. Baum, Z. Kehel, S. Rehman (2016) "**Evaluation of FIGS and GCP subsets for resistance to scald disease (*Rhynchosporium commune*)**". At the 10 AMPP Congress, Rabat, Morocco, November 22-23, 2016 **Oral**

H. Hiddar, A. Amri, A. Filali-Maltouf, S. Rehman, M. Baum, Z. Kehel (2016) "**Evaluation of GCP set for resistance to scald disease (*Rhynchosporium commune*)**". At the American Moroccan agricultural sciences conference IV (AMAS), Agropolis, Meknes, May 9-11, 2016. **Oral**

Abbreviations

ACR: Annoceur station

Adm: Admix

AI: Artificial Intelligence

AM: Association Mapping

AMOVA: Analysis of Molecular Variance

APD: Active Plant Defense

APS: Adult plant screening

AR: Allelic Richness

AUDPC: Area Under the Disease Progression Curve

Avr: Avirulence

BCART: Bagged Carts

BLINK: Bayesian-information and Linkage-disequilibrium Iteratively Nested Keyway

BLUE: Best Linear Unbiased Estimator

BLUP: Best Linear Unbiased Predictor

BMMV: Barley Yellow Mild Mosaic Virus

bp base: pair

BYDV: Barley Yellow Dwarf Virus

BYMV: Barley Yellow Mosaic Virus

CG: candidate Gene

Chr: Chromosome

CWR: Crop Wild Relative

CYDV: cereal Yellow Dwarf virus disease

DH: Double Haploid

DNA: Deoxyribose Nucleic Acid

EST: Expressed Sequence Tag

ETI: Effector Triggered Immunity

FarmCPU: Fixed and random model Circulating Probability Unification

FIGS: Focused Identification of Germplasm Strategy

FIS: Inbreeding Coefficient

FN: False Negative

FP False Positive

FST: Fixation Index

GBS: Genotyping by Sequencing

GCP: Generation Challenge Program

GD: Genetic Diversity

GLM: General Linear Models

GS: Genomic Selection

GWAS: Genome Wide Association Studies

He: Expected Heterozygosity

Ho: Observed Heterozygosity

HS: Highly Susceptible

I: Immune

ICARDA: International Center for Agricultural Research in the Dry Areas

IR: Infection Responses

K: Kinship Matrix

KNN: K Nearest Neighbors

LD: Linkage Disequilibrium

LMA: Lima Bean Agar

LOD: Logarithm of the Odds

LRR: leucine-rich-repeat

MAF: Minor Allele Frequency

MAMPs: Microbe-Associated Molecular Patterns

MAS: Marker Assisted Selection

MAT Matting Types

Mbs: Megabases

MCH: Marchouch station

ML: Machine Learning

MLM: Mixed Linear Models

MLMM: Multiple-locus MLM

MR: Moderately Resistant

MS: Moderately Susceptible

MTA: Marker-Trait-Association

NGS: Next Generation Sequencing

NIP: Necrosis Inducing Proteins

NLR: Nucleotide-binding Leucine-rich Repeat

Npa: Number of Private Alleles

PAMPs: Pathogen Associated Molecular Patterns

PCA: Principal Component Analysis

PEI: Pectin Esterase Inhibitor genes

PIC: Polymorphism Information Content

PRRs: Pattern Recognition Receptors

PTI: Plant Immunity

Q-Q: Quantile- Quantile plots

QTL: Quantitative Trait Loci

R: Resistant

Rc: *Rhynchosporium commune*

RF: Random Forest

RFLP: Restriction Fragment Length Polymorphism

RLKs Receptor Like kinases

RLPs Receptor-Like Proteins

Rrs: Reaction/Resistance to *Rhyncosporium secalis*

RS: *Rhynchosporium secalis*

S: Susceptible

SAT: Sidi Allal Tazi station

SC: Scald

SNMF: Sparse Non-negative Matrix Factorization

SNP: Single Nucleotide Polymorphism

SP: subpopulation

SRT: Seedling screening Types

SSRs: Simple Sequence Repeats

SUPER: Settlement of MLM Under Progressively Exclusive Relationship

SVM: Support Vector Machines

TN: True Negative

TP: True Positive

Contents

List of figures

List of tables

General introduction

Food is regarded as the basic need of human being which can be satisfied through farming. Agriculture not only fulfills humans' basic needs, but is also considered as the backbone of economy. Owing to the fast increase of the world population, the majority of agricultural research and development efforts are devoted to increasing crop production.

Agriculture plays an important role in the balance of the Moroccan economy, with cereals being the predominant field crops and major component of food security. Barley (*Hordeum vulgare* L.*)* is a rustic cereal cultivated on all continents extending to the highest altitudes and latitudes and is known for its agronomic, socio-economic and zootechnical importance, leading to its multiple uses as feed, food and beverages and its consideration as a low-risk aversion crops by farmers. Globally, it was sown over 51 million hectares with a production of 150 million metric tons in 2019 (FAOSTAT., 2020). Barley is an ancient food for many early human civilizations. Earlier, barley is known as hearty tasting, high-energy food. Historically, Hordearii, or "barley men," were the names given to the Roman gladiators because they consumed barley to increase their strength and endurance (Percival., 1921).

Morocco is among the top ten of the world's largest barley growing countries with an average of 1.8 M ha sown annually, and is known for its highest annual per capita consumption as food (more than 35 kg per person) (FAOSTAT., 2005). Among other crops, barley has a number of distinctive characteristics. It has a great level of adaptation, as it can grow in a wide range of environmental conditions (drier environments, saline, moist, and high altitudes) (Dai & Zhang, 2016; Hecht et al., 2016; Serna-Saldivar, 2016). In Morocco, more than 70% of its acreage is located in dry areas, 11% in mountainous areas and often does not benefit from inputs. However, barley quality and productivity are affected by various phytopathogens causing 20–50 % of total global agricultural losses (Bellard et al., 2012; Oerke, 2006; Savary et al., 2012). Scald, also known as leaf blotch or *Rhynchosporium*, caused by a haploid hemibiotrophic fungus *R. commune* (formally known as *Rhynchosporium secalis*) is one of the most serious and destructive fungi, globally (X. Zhang et al., 2020). Scald disease is found in all continents, but is more serious and destructive under semi-humid and cool barley growing regions (Zhan et al., 2008a). Economic losses caused by scald disease worth £7.2 million in UK despite the fungicide treatment (Paveley et al., 2016). Scald disease management and control is difficult and more challenging, due to the high genetic diversity of the pathogen population and its great potential to quickly adapt to abiotic selection pressure, comprising the global warming and variation of temperature (Stefansson et al., 2013). In addition, *R. commune* population has

quickly developed resistance to multiple fungicides (Brunner et al., 2016; Mohd-Assaad et al., 2016).

Genetic resistance remains the most efficient, environmentally friendly, and economical strategy to control *Rhynchosporium* disease, but requiring continuous search for new genes of resistance. Genetic diversity is the basis of any crop improvement program. However, in the last decades, a large part of the genetic diversity available to breeders has been eroded due to the strong selection pressure imposed through genetic improvement (Jing et al., 2013b), leading to a reduction in allelic plasticity, hence to varieties less adapted to new environmental stresses, diseases and pests (Makai et al., 2016; Tanksley & McCouch, 1997). To maintain genetic diversity while breeding cultivars that combine biotic resistance with high yields is a prerequisite for stable productivity in the Mediterranean climate. Compared with adapted genetic materials, wild relatives and landraces have been challenged in natural environments for thousands of years and maintain a much higher level of diversity (Zhang et al., 2017).

Plant breeders and scientific researchers are utilizing wild plant relatives and landraces along with genetic stocks stored in gene-banks, and breeders constructed panels to discover new sources of resistance. Resistance to scald disease has been identified from multiple sources, including the cultivated barley species, *Hordeum vulgare ssp. vulgare* (Hofmann et al., 2013; Silvar et al., 2010), the wild barley, *Hordeum vulgare ssp. spontaneum* (Von Korff et al., 2005a; Yun et al., 2005), and from *Hordeum bulbosum* as explored by Pickering et al. (2006). Thus, genetic resource collections conserved in the gene-banks are the most obvious place to find new genes for useful traits. The gene bank of the International Center for Agricultural Research in the Dry Areas (ICARDA) has one of the third largest collections of barley in the world totaling around 32,800 accessions, 60% of which are landraces and 7.6% are from wild *Hordeum* species (*Genesys.*, 2018).

Owing to the large size of the genetic resource collections, discovering useful traits for crop improvement is more challenging and is like searching a needle in a haystack. In addition, it can be very expensive to evaluate huge collections for some adaptive traits.

However, to enhance the discovery of new genes for resistance to biotic stress within a large number of accessions, ICARDA joined efforts with Australian and Russian collaborators to develop a new approach for efficient mining of gene-bank accessions, known as Focused Identification of Germplasm Strategy (FIGS). This approach uses predictive modelling or filtering to identify accessions with sought trait through the development of appropriate algorithms. FIGS approach allows to develop algorithms linking the sought plant traits to climatic, edaphic and geographic variables, and construct manageable best-bet subsets for

targeted traits (Azough et al., 2019; Mackay et al., 2004). Several studies have demonstrated the effectiveness and the success of FIGS approach in identifying sources of resistance to several biotic stresses including for resistance to powdery mildew (Bhullar et al., 2009), Russian wheat aphid and Sunn pest (El Bouhssini et al., 2009, 2011) in wheat, and net-blotch in barley (Endresen et al., 2011). Another approach was developed through the Generation Challenge Program (GCP), in the aim to keep within a minimum number of accessions, the maximum diversity in 10% of the core collection in a "Reference subset" using molecular markers.

Plant breeding has progressed significantly since its inception, experiencing remarkable advancements in recent decades genomics and bio-informatics. Cutting-edge technologies such as high-performance computing, bioinformatics tools, artificial intelligence (AI), and machine learning (ML) are being harnessed for the analysis large data sets in gene-banks and breeding programs (Yoosefzadeh-Najafabadi et al., 2021).

In the agricultural sector, the incorporation of machine learning (ML) into plant breeding is an emerging trend offering the agricultural industry more economically viable and streamlined approaches to developing novel crop varieties (Yoosefzadeh-Najafabadi et al., 2021).

With the help of the advanced technologies in computer science, machine learning (ML), which is a branch of artificial intelligence (AI), is considered a powerful tool that can make plant breeding more accurate, efficient, and capable of evaluating a wider set of variables (Libbrecht & Noble, 2015). Using computer simulations, it allows breeders to assess different predictive algorithms for efficient mining of genetic resources for resistance to diseases. Instead of simply following static program instructions, these algorithms operate by creating a model from sample inputs in order to generate data-driven predictions or decisions (Bishop., 2006).

There are different machine learning algorithms used for prediction including, K-nearest neighbors (KNN) (Kotsiantis et al., 2007), Support Vector Machines (SVM) (Hsu et al., 2003), Random Forest (RF) (Breiman, 2001), and Bagged Carts (BCART) (Kołcz et al ., 2000).

The past two decades have also witnessed important progress in developing and deploying plant genomics platforms to identify agronomically valuable loci, in order to improve selection in crop improvement. These areas of research revolve around harnessing the power of genomic data to elucidate the genetic underpinnings of specific traits, ultimately fueling advancements in plant breeding. The process entails identifying these favorable traits and subsequently incorporating them into breeding programs (Li & Yan, 2020). The utilization of genetic

sequencing techniques has provided researchers with the means to pinpoint genes linked to significant traits like disease resistance.

The recent progress and increased availability of high throughput genotyping technologies (Comadran et al., 2012a; Moragues et al., 2010), the availability of a high-quality reference genome assembly (Mascher et al., 2017), and the improvement of statistical programs have significantly contributed to the genetic dissection of complex traits into QTLs. The disease resistance controlled by major genes or minor genes can be effectively and efficiently detected using molecular marker techniques through genome wide association studies (GWAS).

Genome-wide association studies (GWAS) have been recognized as a highly effective method for capturing a broader range of genetic variation and providing more precise mapping of quantitative trait loci (QTL) and their related candidate genes, surpassing the capabilities of classical linkage analysis (Bayer et al., 2017; Cockram et al., 2008; Comadran et al., 2012a). The identified markers can be used in efficient selection for resistance and in pyramiding major and minor genes of resistance to ensure durable resistance (Walters et al., 2012).

Aims of the study

Barley as many crops is affect by various phytopathogens in consequence, the identification of new sources of resistance to cope with changes in virulence spectrum of the disease and the search for linked markers and genomic regions are crucial for the development of high yielding and scald resistant barley varieties.

This study aimed at :

1. The identification of sources of resistance to scald in diverse genetic resources subsets (FIGS, GCP, *Hordeum spontaneum*) and germplasm included in ICARDA barley breeding panel (AM17).
2. Comparing GCP and FIGS subsetting approaches in identifying sources of resistance to scald and using artificial intelligence to assess different predictive models for efficient mining of genetic resources for resistance to scald.
3. Identify genomic regions associated with resistance to scald in these subsets using Genome Wide Association Studies.

Chapters description

Chapter I- Literature review: This chapter represents generalities about barley, its global status, scald disease resistance and application of machine learning algorithms and GWAS approach.

Chapter II- Assessment and modeling using machine learning of resistance to scald (*Rhynchosporium commune*) in two specific barley genetic resources subsets: In this chapter, we assessed the efficiency of FIGS approach in comparison with GCP. This work was published in Nature scientific reports in 2021.

Chapter III- Identification of sources of resistance to scald (*Rhynchosporium commune*) and of related genomic regions using Genome-Wide Association in a mapping panel of spring barley: This chapter presents the results of a genome wide association study of association mapping panel (AM17) under both plant growth stages. QTLs related to scald disease resistance were assessed. This work was published in Frontiers journal in 2023.

Chapter IV- Identification of sources of resistance to scald (*Rhynchosporium commune*) and of the associated molecular markers in a set of accessions of *Hordeum spontaneum* genetic resources: This chapter presents the results of genetic diversity and genetic structure on wild barley *Hordeum vulgare* subsp. *spontaneum subset* collected from different parts of the world and conserved at ICARADA gene bank and tested against *Rhynchosporium commune* at both plant growth stages. It also includes the results of GWAS analysis. The paper is drafted for submission to publication.

Chapter V- General Discussion and Perspectives: This chapter presents a general discussion and main conclusions along with perspectives to guide future research.

Chapter I: Literature review

I.1 Barley crop features

Barley *(Hordeum vulgare. L*), is one of the earliest domesticated cereals, while switching from gathering and hunting to cultivation of land and animal husbandry (Badr et al., 2000; Harwood, 2019; Wang et al., 2015). Barley has many unique features, it is known for its high adaptation to different climatic and geographical conditions, varying from deserts, to highest latitudes and altitudes (Dai & Zhang., 2016; Verstegen et al., 2014). Several studies concluded that there is a prevalent consensus that barley is more stress-tolerant than wheat and is an excellent model for studying responses to stress conditions as those expected with climate change (Dawson et al., 2015; Newton et al., 2011; Ryan et al., 2008). Due to easy cultivation, availability of high-quality reference genome sequence, availability of extensive genetic resources, diploid genome (2n = 14), and easy pollination techniques, barley has become a model crop for a range of applications including cytogenetics, genetics, plant breeding methodology, virology, pathology, and biotechnology studies (Harwood., 2019; Lightfoot & Able., 2010; Verstegen et al., 2014). Cultivated barley (*Hordeum vulgare* sbsp. *vulgare),* is one of 31 *Hordeum* species, member of the *Poaceae* grass family also known as Gramineae, and belonging to the tribe Triticeae. It's a diploid and self-pollinating annual species, which in contrast to competitors like rice and wheat, requires a minimal amount of fertilizer (Kant et al., 2016; Singh et al., 2019). It is thought that barley was first domesticated and is supposed that cultivated barley *Hordeum vulgare L.* was derived from *H. spontaneum* C. Koch, the only wild *Hordeum* species that can be easily crossed with *H. vulgare* (Verstegen et al., 2014) while crosses with *H. bulbosum* are more difficult.

Around the globe, barley is grown in both spring and winter season, with winter annuals being planted in autumn and requiring a period of vernalization before they will flower (Cuesta-Marcos et al., 2015). In addition, it is differentiated by variation in spike morphology and hull types (Harwood., 2019). Ancient barley was originally two-rowed featured with high-quality grain and was sown in spring for direct human consumption. The second is called six-row type because it grew rapidly in low and erratic rainfall areas but modern barley cultivars vary in spike morphology, with both two-row and six-row types (Horsley et al., 2009).

I.2 Barley production and utilization

I.2.1 Barley production

Barley is a robust crop and is grown in more than 100 countries around the world (Harwood., 2019). According to the Food and Agriculture Organization Corporate Statistical Database, it is among the four major crops produced worldwide (FAOSTAT., 2020), with a global production of 147.05 million metric tons in the 2021/2022 crop year, decreasing from around 160.53 million metric tons in 2020/2021 (FAOSTAT., 2020). Globally, the European Union was the largest producer of barley, with approximately 53 million metric tons of barley production, followed by Russian Federation which is the second largest producer, with 17.5 million metric tons produced in 2020/2021 (Figure I.1) (Statistica., 2021). Russian Federation, Germany, France, Canada, Ukraine, Spain, Australia, Turkey, United Kingdom of Great Britain and Northern Ireland are the major barley-producing areas in the world (Figure I.2) (FAOSTAT., 2021).

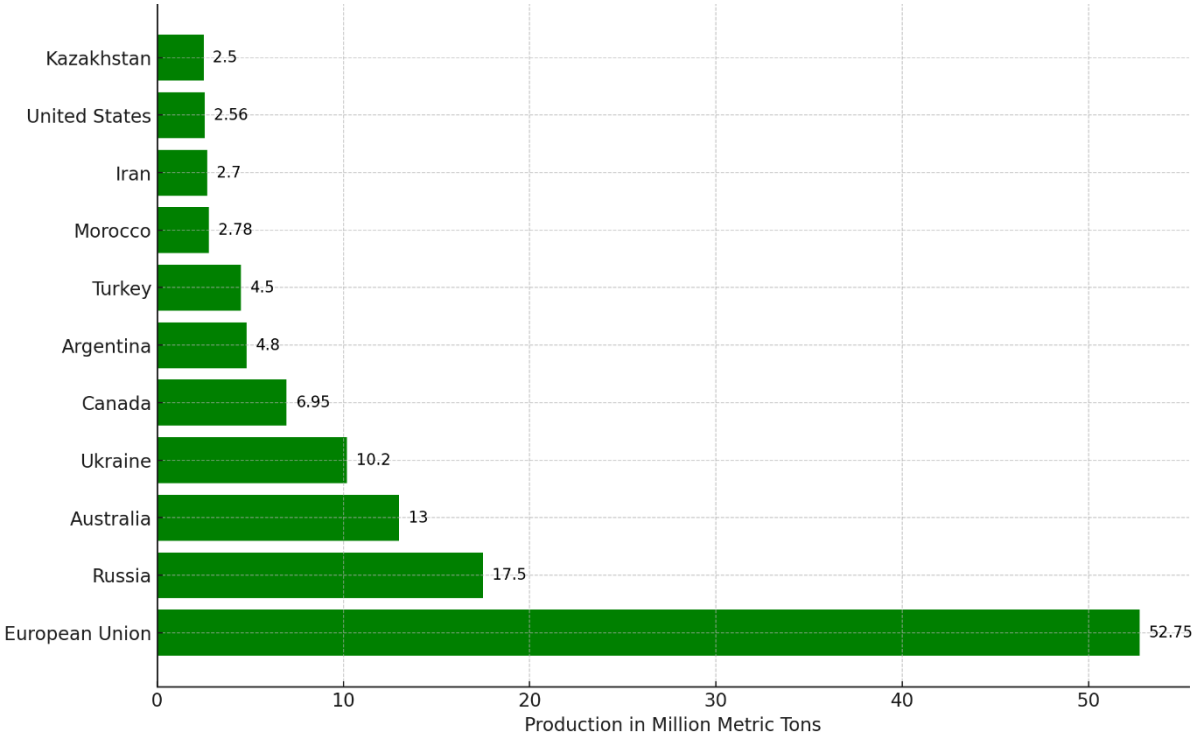


Figure I.1. Major barley producers 2021/2022 (Statistica., 2021)

The top five barley-exporting countries worldwide in 2021/2022 crop year, were European Union, Australia, Ukraine, Russia and Argentina. Australia was the largest exporter with 8.5 million metric tons of barley followed by the European Union with approximately 7.3 million metric tons (Figure I.2).

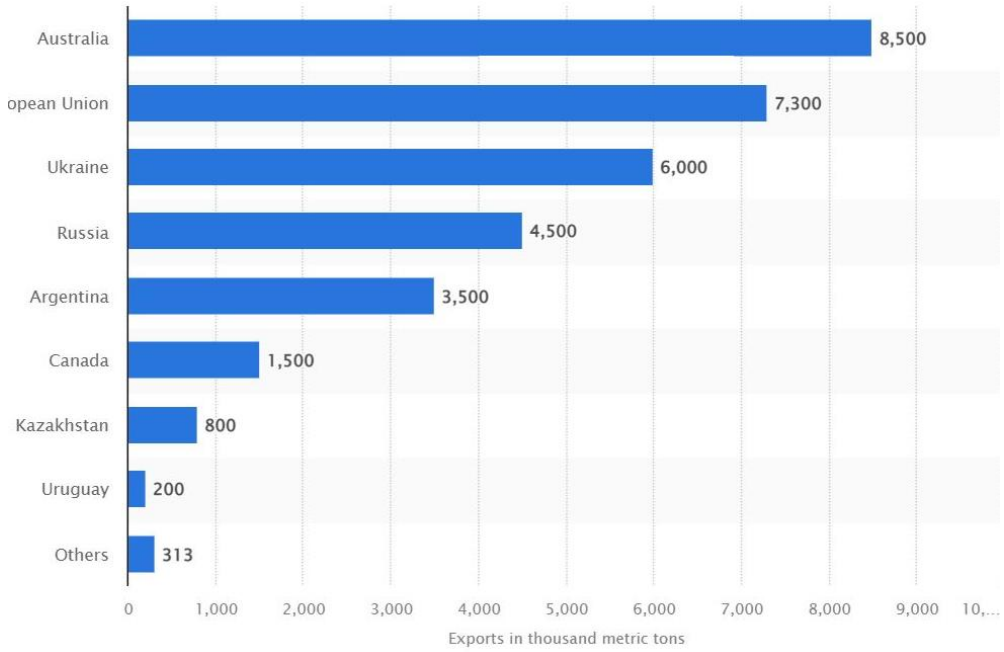


Figure I.2. Major barley exporters in 2021/2022 (in 1,000 metric tons)

In North Africa and West Asia, barley is considered a major crop, with highest acreages recorded in Morocco, Türkiye and Ethiopia with more than 1.5 M ha sown annually in each country (FAOSTAT., 2023). In Morocco, barley occupied around 13% of the total cereals production with an average of 2 million tons in the last ten years. In the 2021-2022 crop year, Morocco imported about 9.4 million quintals of barley. This was an increase of barley importation compared to the 2020-2021. Annually, barley covers more than 2 million hectares with 70% in the arid and semi-arid regions that are known for their harsh conditions with annual rainfall of 80–350 mm (Jebbouj & El Yousfi., 2010). Due to the breeding efforts, high yielding and disease resistant varieties have allowed an increase in yield of 55% in the past two decades in Europe, the United Kingdom, and Turkey, when applying appropriate agronomic practices (Friedt et al., 2011; Harwood., 2019).

I.2.2 Barley utilization

Barley is a multiple purpose crop used for feed (green forage, straw, and grain), food, malt, and brewing. The entire plant, including the fruit kernels, spike, whole plant for ensilage, and straw, is typically used (Harwood., 2019). Historically, barley has been a major component in household food security and an important feed source. In the beginning of the second millennium and since the “Mourabitine” dynasty, the population has based its food security and safety by using a large storage facilities “Makhazen” mainly reserved for cereals.

In many developed and developing countries barley is an important food of the modern human diet (Ullrich., 2010). However, more than 90% of barley is used as human food in Japan (Aoki et al., 2011). Further, barley grain remains widely consumed as an important ingredient in Morocco, Ethiopia, India and China (Ashman & Beckley., 2006). Barley is considered as a staple food is still consumed in mountainous areas of Central Asia, Southwest Asia, and Northern Africa.

Barley is not only limited to feed and food, owing to its high nutritional value and health benefits barley uses are extended as one of 300 species being used in Chinese herbal medicine (Zeng et al., 2018). Several studies have highlighted its potential to prevent or treat chronic diseases, including (diabetes, cancer, obesity, cardiovascular disease, lowering cholesterol) (Baharvand-Ahmadi et al., 2016; Hong & Jai Maeng., 2004; Park et al., 2022; Poonthananiwatkul et al., 2015; Woo et al., 2017; Zeng et al., 2018). Further, barley is also for the production of biofuels (Griffey et al., 2010).

I.3 Barley diseases

Barley production worldwide is limited by several biotic stresses and breeding of highly productive and adapted varieties is key to overcome these challenges. Globally, disease resistance is the primary priority for all barley breeders (Barati et al., 2018; Ceccarelli et al., 1992; Francia et al., 2011).

The high level of barley adaptation has increased the range of phytopathogens invasion. These phytopathogens include bacteria, viruses, and fungi (Pessarakli., 2019). Further, there are more than 250 phytopathogens attacking cultivated barley and affecting yield and quality. Each pathogen has a specific symptom and targets a particular organ or a specific developmental stage (https://cereals.ahdb.org.uk/). However, the disease incidence is depending on different factors including phytotoxin synthesis, prevailing agricultural practices, geography, plant age, local climate, and soil type. To date, the most economically and devastating diseases are caused by fungi infection including scald/leaf blotch (*Rhynchosporium commune*); net blotch (*Pyrenophora teres*); powdery mildew (*Blumeria graminis*); head blight (*Fusarium sp*.); speckled leaf blotch (*Septoria passerinii*); barley yellow dwarf virus (BYDV); smut (*Ustilago sp*.); barley yellow mild mosaic disease (BaMMV); yellow rust (*Puccinia* sp.); barley rusts like brown rust, leaf spot (*Ramularia sp*.); barley yellow mosaic disease (BaYMV); cereal yellow dwarf virus disease (CYDV); and black rust. Further, cultivated barley is a host of a number of pests, particularly worms, aphids, and beetles (Singh et al., 2019).

I.4 Barley gene pools

The crop genetic diversity was categorized by Harlan & de Wet in (1971) into primary, secondary, and tertiary gene pools, which have less taxonomic significance but are helpful for utilizing the diversity in crop improvement. Although there was a sterility barrier, it was previously believed that all species in the genus *Hordeum* were related and together they formed genetic resources for breeding. These species are separated into various gene pools according on how closely related to cultivated barley they are, how easily they can cross, and how easily they can transfer genes (Figure I.3).

I.4.1 Primary gene pool

The primary gene pool concludes landraces, breeding lines, the ancestor of cultivated barley, *H. vulgare L.* spp. *spontaneum.* It is possible to cross primary gene pool species without obstacles and barriers, and the resulting hybrids are typically viable and fertile and have adequate chromosome pairing (Harlan & de Wet., 1971). This gene pool contains several desirable features, especially genes for disease resistance. The hybrids' gene segregation is roughly normal; thus, genes can be transferred without barriers (Harlan & de Wet., 1971). Barley breeding has frequently benefited from the diversity provided by landraces (von Bothmer et al., 2003).

I.4.2. Secondary gene pool

The secondary gene pool contains only one species *Hordeum bulbosum L.* for which the transfer is possible but difficult (von Bothmer., 1996). The Mediterranean basin and the Fertile Crescent are both within the extensive geographic range of *H. bulbosum* (von Bothmer., 1996). *H. bulbosum* includes perennial diploid and tetraploid species that have shown crossability despite sterility factors and the selective elimination of *H. bulbosum* chromosomes from the hybrid.

I.4.3 Tertiary gene pool

The tertiary gene pool of barley comprises 31 species (von Bothmer., 1996). These include species that are self-pollinating, out-breeding, annual, perennial, diploid, tetraploid, and hexaploid (von Bothmer., 1996). Because of the tertiary gene pool's distance from the primary gene pool, inter-pool crossing is prohibited. Due to the large genetic differences between these species and cultivated barley, crossbreeding between these species and cultivated barley is challenging. To date, this bottleneck has prevented the germplasm from this gene pool from advancing barley breeding. However, the transfer of traits to the primary gene pool may become possible with the development of new techniques, such us embryo rescue, the fusion of

protoplasts, chromosome doubling or the use of other species as a bridge for crossing (bridge crossing) (Harlan & de Wet., 1971). Further, research efforts were able to create hybrids through protoplast fusion between barley and species of other genera such us *Elymus, Thinopyrum and Pseudoroegneria* (Kim et al., 2008), rice (Kisaka et al., 1998), rye (Fedak & Armstrong., 1981) and even of different plant families like carrot (*Daucus carota*) (Kisaka et al., 1998), soybean (Kao et al., 1974) and tobacco (*Nicotiana tabacum*) (Somers et al., 1986). However, the majority of the hybrids that were produced were sterile, and therefore have not contributed to the enhancement of barley. Geographically, the tertiary gene pool species have a wide geographic range and are indigenous to both the northern and southern hemispheres, including Central Asia, South Africa, Europe, the Middle East, North America, and South America (von Bothmer., 1996). In nature, hybridization between the third gene pool species and barley do not occur.

I.4.4 Quaternary gene pool

In modern terminology, it was suggested that isolated genes should be included in the definition of genetic resources (Becker., 1993). Therefore, genes from distant plant species, from animals and from even micro-organisms can be transferred using transformation technology, making them the fourth gene pool (Becker., 1993). Dunwell in (2000) and Phipps in (2002) indicated that the fourth gene pool can significantly contribute to crop development. Furthermore, due to high regulatory challenges and a low level of consumer acceptance, particularly in Europe, genetically modified barley cultivars are not currently being used commercially (Verstegen et al., 2014).

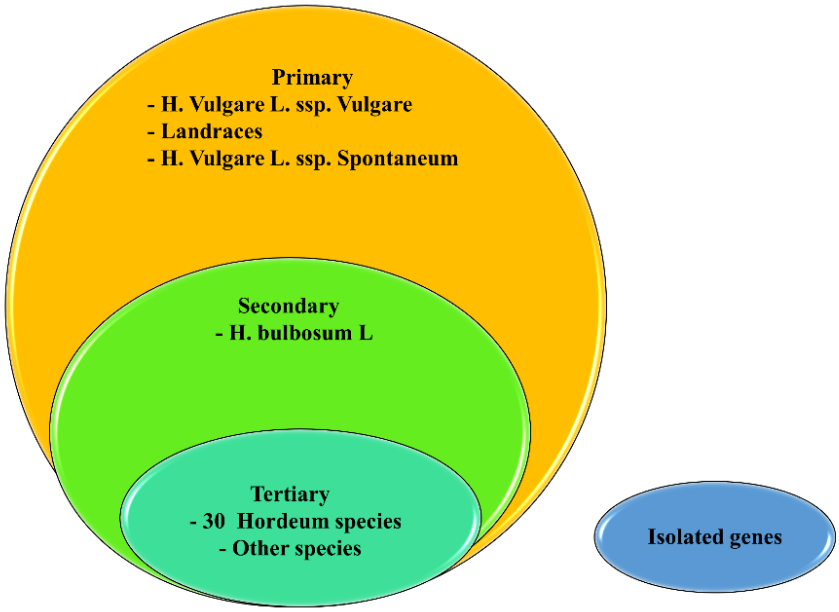


Figure I.3. Gene pool concept for barley (Becker., 1993; Harlan and de Wet., 1971)

I.5 Genetic and genomic resources of barley

I.5.1 Genetic resources

The genetic resources of a crop are important raw material for both plant breeding and scientific research. To meet the requirement of various types of products, diverse germplasm for the development of product-specific varieties is needed. Becker in (1993) defined genetic resources as "all materials that are available for improvement of a cultivated plant species". Based on the gene pool concept, genetic resources can be classified into three categories, referred to as the primary, secondary, and tertiary gene pools (Harlan & de Wet., 1971).

Barley has become a more important source of raw materials for the malting and brewing industries in recent years. However, the fast spread of improved crop varieties throughout the world has replaced many of the genetic resources necessary for their continuous improvement and could constitute a serious danger to the global barley production. The basic purpose of any breeding program is the development of cultivars that are stable, productive and with high-quality. Successful breeding program is strongly attributed to sustainable utilization of genetic diversity. Consequently, collecting, evaluation and maintaining genetic resources have become a priority area in breeding programs across the world, and making them available is key to respond to this objective.

More than 70 years ago, Nikolai Ivanovich Vavilov started the collecting and conservation of plant genetic resources in order to preserve genetic resources and study the ancestry of cultivated plants (Vavilov., 1940). The most significant and impressive uses of genetic resources in plant breeding were seen after 1960. Norman Borlaug introduced dwarfing genes providing insect resistance and durable disease resistance into wheat, promoting the Green Revolution (Rajaram., 1995). It is crucial to give high priority to the assessment and evaluation of the germplasm for identification of the donors for different useful and desirable traits (Kant et al., 2020).

I.5.2 Conservation of genetic resources

There are two methods for genetic resources conservation, the ex-situ conservation referring to the preservation of genetic resources away from their natural habitat in seed or field gene banks and the in-situ conservation referring to the preservation of genetic resources in their natural habitats or field conditions.

I.5.2.1 Ex situ conservation

The most significant and widely used method of preserving genetic resources is ex situ conservation, mainly in the form of seeds in genebanks.

Barley collection is regarded as the second-largest of cereals, after wheat. More than 200 different genebanks worldwide are conserving more than 485,000 accessions for the genus *Hordeum* (FAOSTAT., 2010). These collections contain 299,165 accessions of *H. vulgare* ssp. *vulgare* (mostly new and old cultivars and landraces), 32,385 accessions of *H. vulgare ssp. spontaneum,* 4,681 accessions of wild *Hordeum* species, and a sizable representation of genetic stocks, breeding lines, and mapping populations (Knüpffer., 2009). In order to ensure safety or to avoid quarantine issues, many accessions are duplicated between gene banks. The Svalvard Seed Vault, which preserves approximately 1 million crop-related accessions, including 92 075 *Hordeum* accessions is the largest seed storage facility in the world as of July 2020 (https://www.nordgen.org/sgsv/). ICARDA has the second largest barley collection with 32 800 accessions including 2500 accession of wild *Hordeum* species.

I.5.2.2 In situ conservation

In situ and ex situ conservation are complementary. Unlike ex situ conservation, which is static, this method of conserving germplasm is active. By letting natural selection take effect, it enables the continuous evolution of barley. Recently, in situ conservation has received a lot of attention, and attempts are being made to preserve the genetic resources in their natural habitat. It is crucial for the conservation of species that are challenging to maintain in ex situ conditions, particularly crop wild relatives (CWR). Crop wild relatives are playing a crucial role in crop genetic improvement efforts as new biotechnological techniques develop. Unlike ex situ conservation, in situ conservation frequently occurs in protected regions or habitats (Kant et al., 2020). Landraces of barley are still prevailing in the traditional farming systems and in mountainous and arid regions and efforts are devoted to promote their farm conservation through technological, add-value, alternative sources of income and institutional options (Mazid et al., 2013).

- **Utilization of wild barley in breeding**

Barley genetic resources, particularly those related to wild barley, are a rich source of diversity that has not been fully studied or used due crossability barrier (Brown et al., 1978; Nevo., 2015) The CWR of barley are a source of many agronomically important traits such as tolerance to abiotic stresses including (drought, cold, heat, salt, and low mineral), and resistance to biotic stresses including (virus, bacteria, fungus, and weeds), dormancy, and high-quality for malting and feeding that can be transferred from wild barley to cultivated (Zhang et al., 2005; Yan et

al., 2008). However, there are unexploited sources of unique disease-resistance genes, yield attributes, and quality in *H. vulgare* ssp. *spontaneum* and *H. bulbosum* species.

➢ *H. vulgare* ssp. *spontaneum* as a resource for barley improvement

Hordeum vulgare subsp. *spontaneum* is the wild progenitor and the only wild species in the primary gene pool. Wild barley *Hordeum spontaneum* (L.) shows a wide geographic distribution and ecological diversity (Bedada et al., 2014). The wild barley *(H. vulgare* ssp. *spontaneum)* is also cross-compatible with wheat and can be used to introduce agronomically important traits (Taketa et al., 2008).

Due to advancements in research, *H. vulgare* ssp. *spontaneum* was successfully used for breeding purposes, which is known by it is highly richness of unique alleles for resistance, yield components, and nutritional quality. This has also been investigated for developing an efficient molecular marker system (Von Bothmer & Komatsuda., 2011). In addition, due to its winter annual nature, this has also been employed to provide permanent pasture for grazing in some regions (El-Shatnawi et al., 2004). Wild barley (*Hordeum spontaneum*) offers great potential as source of resistance compared to landraces. Several studies have demonstrated that *H. spontaneum* accessions have sources of resistance/tolerance to major abiotic and biotic stresses (Azamparsa & Karakaya., 2020; Von Korff et al., 2005). It has been enabled to effectively introduce resistance genes from *H. spontaneum* into cultivated barley, which offers improved defense against various diseases and pests. This comprises defenses against powdery mildew (Jørgensen., 1992; Moseman Nevo, & Zohary., 1983), scald (Abbott et al., 1991), leaf rust (Steffenson et al., 2007), and the aphid Rhopalosiphum padi (Åhman & Bengtsson., 2019). Further, Grando et al., in (2005) developed a marker system for the nutritional quality of straw that is being enhanced in cultivated barley by the introduction of genes from *H. spontaneum.*

➢ *Hordeum bulbosum* as a resource for barley improvement

After *ssp. spontaneum*, *H. bulbosum* is the second-closest related to cultivated barley and is a member of the secondary gene pool. *Hordeum bulbosum* plays a crucial role for barley improvement and is considered as an important source of resistance to disease and insect pests. It has successfully been investigated to transfer genes for resistance to diseases caused by scald (Pickering et al., 2006; Singh et al., 2004), leaf rust, and mosaic (Walther et al., 2000), and soil-borne viruses (Pickering et al., 2006). *H. bulbosum* is believed to be a source of durable resistance since it presumably possesses non-host resistances to a number of barley pathogens (Johnston., 2007). Selective chromosome elimination is another significant *H. bulbosum* feature

that has been extensively used in barley and wheat breeding to create double haploid plants (DH). Strong crossability barriers between barley and *H. bulbosum* prevent the development of fertile hybrids, which is the first stage in trait introgression. Cross-fertilization between *H. vulgare* and *H. bulbosum* typically results in the elimination of *H. bulbosum* chromosomes during the first mitotic divisions, leaving a haploid barley embryo (Kasha & Kao., 1970). Diploid hybrids that contain one haploid copy of the barley and *H. bulbosum* genome, are completely sterile, but fertility can be restored if the young plants are treated with colchicine (Pickering., 2000). However, this method was replaced by microspore culture and anther techniques (Pickering & Devaux., 1992).

I.5.3 Genomic resources of barley

Rapid identification of the genes responsible for useful traits including yield, malting quality, and pest and disease resistance has been made possible by the availability of enhanced genomic resources and analysis tools in barley. There are many genomic resources available, including a high-quality barley reference genome sequence. Barley has a relatively big genome, with at approximately 5.3 Gbp with > 80% of repetitive elements (Mascher et al., 2017). More than 39 000 genes are found at the 5.3 Gbp barley genome (Harwood., 2019). About 30,000 genes were found to belong to gene families with multiple members, and it was revealed that annotated transposable elements made up 80.8% of the genome sequence (Mascher et al., 2017).

To analyze complete or partial genomic sequences and their associated functions in barley, numerous genomic resources have been developed. Since 2000, several large barley expressed sequence tag (EST) projects have generated large numbers of sequences from expressed genes (http://harvest.ucr.edu/). Using traditional three-point linkage tests, barley geneticists have created high-quality genetic maps based on mutant phenotypes (Horsley et al., 1997). Genome-wide genetic maps derived from molecular markers are being used to update and supplement these efforts. With 1,032 EST-based loci analyzed using a variety of marker assays, Stein et al in (2007) created a consensus barley map. Further, with sing a single-mapping population, Sato et al., (2009) have developed a high-resolution barley EST map with 2890 loci. The allele-specific detection of SNPs is a component of high-throughput and high-quality multiplex PCR-based genotyping assays based on Golden Gate technology (Illumina Inc., CA, USA). A consensus genetic linkage map with over 2,900 gene-based SNP markers (http://harvest.ucr.edu/) was developed (Close., 2009; Muñoz-Amatriaín., 2011).

➢ **Genomic information and available databases**

Several databases are available pertaining to barley genomic and genetics. Table I.1 illustrates the lists of databases of barley and their functions. IPK allows the user to conduct BLAST searches against all sequence resources published by the worldwide barley community, including activities related to the International Barley Sequencing Consortium. For genome assembly and annotation, EnsemblPlants provides easy access to the most updated barley genome assembly, including chromosome sequences, genes, transcripts, and predicted proteins. The same website also supports Basic Local Alignment Search Tool (BLAST) searches against the barley genome.

Table I.1: Barley genome databases

Name	URL	Function
EnsemblPlants	http://plants.ensembl.org/Hordeum_vulgare	Browser, BLAST
IPK (IBSC) Barley BLAST server	https://webblast.ipkgatersleben.de/barley_ibsc/	BLAST
PLEXdb	http://www.plantgdb.org/prj/PLEXdb/	Gene expression analysis
HarvEST	http://harvest.ucr.edu/	cDNA sequence
barleyGenes	https://ics.hutton.ac.uk/barleyGenes/	RNA-seq data
bex-db	https://barleyflc.dna.affrc.go.jp/bexdb/	cDNA, gene expression
GrainGenes	http://www.graingenes.org	Markers, maps, mutants, etc.
Barley DB	http://www.shigen.nig.ac.jp/barley/	Seed collection, cDNA sequence

I.6 Genebanks strategies to improve breeding for biotic stress

Globally, the gene banks hold collections of a broad range of plant genetic resources, including landraces, obsolete cultivars from different agroecologies and crop wild relatives (CWR). These genetic resources provide diverse novel alleles for various significant traits. in order to maintain plant genetic resources for the long-term and to make the germplasm accessible to plant breeders, researchers, and other users. Thus, gene banks are the most obvious place to look for useful traits, however due to the large size of these collections, we cannot afford to evaluate each accession in gene bank and therefore identification of specific traits is highly difficult, complicated and like searching for a needle in a haystack. In addition, evaluating large collections can be extremely costly. The International Center for Agricultural Research in the

Dry Areas (ICARDA) houses a globally important collection of over 32,800 barley accessions including more than 2500 accessions of wild *Hordeum* species (Genesys., 2020). Several approaches have been proposed for mining gene bank holdings, overall, these approaches aim to identify gene bank accessions containing agronomic traits of interest.

In the past, accessions have either been distributed randomly or through core collections, with the latter aiming to include 90% of the diversity in just 5% to 10% of the entire holding (van Hintum & Van Soest., 1997).

The core collection was suggested as a technique to work with fewer accessions that would capture "the genetic diversity of a crop species and its relatives with a minimum of repetitiveness." (Frankel., 1984). There are several models of core collection development methodologies (Hodgkin et al., 1995), which in practice it is common to minimize the size of the subset to 10 % of the total size of the original collection (Brown et al., 1989). The Generation Challenge Program (GCP) suggested the development of a reference set employing molecular markers to represent the majority of the genetic diversity present in 10% of the core collection. Whereas core collections are useful for sampling, they are not extremely helpful for identifying plants with the desired traits (Khazaei et al., 2013). To effectively provide the breeding community with this variety in genebanks, the idea of a "Focused identification of germplasm strategy" (FIGS) was developed. The emergence of novel abiotic and biotic threats in the context of climate change led to the search for rare adaptive traits. For example, this strategy was first used to improve South Australian wheat by supplying wheat germplasm with boron tolerance (Endresen et al., 2010). Passport data affiliated with the accessions contain information on morphological traits and the collection site (of late, genomic data). The features of these geo-referenced accessions match the climate factors found in FIGS (Street et al., 2016). As the name indicates, FIGS use information about the target trait's natural selection to help identify samples of accessions that are likely to carry desirable features.

The Focused Identification of Germplasm Strategy (FIGS), is an innovative approach developed to improve the efficiency with which specific adaptive traits are identified from genetic resource collections. FIGS approach is based on the premise that adaptive traits displayed by an accession will reflect the selection pressures of the environment from which it was originally collected (Mackay et al., 2005). FIGS is either based on filtering or predictive modeling, to construct best-bet subsets for targeted traits (Mackay et al., 2004), both are based on relationships between environmental conditions and the sought traits.

The domestication of current crops was significantly helped by the process of plant evolution. The main characters in the FIGS are evolutionary signatures, which are still present in natural environments. Moreover, when historical selection, adaptive processes, ecological processes, and geographical processes are taken into account all at the same time and place, the evolution of plants can be evaluated. Developing a deeper comprehension of these components aids in the modeling and strategy in FIGS. In reality, these variables serve as filters for accurately identifying promising germplasm. In addition, (FIGS) significantly decreases resource usage by helping in the identification of potential genotypes based on environmental data that are likely to harness desired variation (Stenberg and Ortiz., 2021). Phenology features and collection environment/location are included in an accession's passport information, which machine-learning algorithms can utilize to predict accession with particular adaptive traits (Wang et al., 2017; Khazaei et al., 2013). Furthermore, FIGS uses geographic information System (GIS) data and machine learning algorithms to filter out individuals based on various attributes (Khazaei et al., 2013; Azough et al., 2019). FIGS takes into account modelling historic natural selection underlying the adaptive evolution of traits as a result of selection pressures imposed by a given environment. By combining large genotypic data with phenotypic data, it is possible to select a specific individual or discover novel alleles. Thus, integration of genomics with FIGS approach makes the strategy more effective and accurate. Machine-learning algorithms for gene prediction based on genomic sequences are already in place due to the availability of sequencing data across species (Mahood et al., 2020; Chan et al., 2017; Pertea and Salzberg., 2002). Additionally, prediction models are in place which employ environmental and genotypic data predict a genotype's breeding value (Tanaka et al., 2021). In order to select soybean accessions, Jarquin et al in (2016) evaluated genomic prediction using historical data and found that it performed successfully. However, still possible that not all of the target traits have experienced selection pressure and, as a result, they don't all associate well with historical selection (Damián et al., 2020). Therefore, in order to fully understand the geographic distribution of a species, it is crucial to take into consideration nonadaptive evolutionary processes as genetic drift and gene flow (Appiah-Madson et al., 2022; Stenberg and Ortiz., 2021). Modeling might incorporate the potential of available genomic data to interpret gene flow and genetic drift (Bataillon et al., 2022; Gompert et al., 2021). Hence increasing FIGS's efficiency. FIGS can be applied to modern plant breeding techniques like genomic selection to offer additional information and enhance prediction accuracy (Anilkumar et al., 2022). Kehel et al in (2020) attempted to extend FIGS with genomic best linear unbiased predictors (GBLUP) (FIGS+) for the predictive characterization of seed morphometric features

in wheat. By applying the FIGS, Bhullar et al. (2009) sampled 1320 landraces from 16,089 wheat accessions and employed a hierarchical selection technique to identify seven novel functional alleles for powdery mildew resistance. There was not an application of the FIGS approach. Recently, Ariani et al in (2022) have employed the Geographic Agronomic Molecular Approach (GAMA) to incorporate climate data, agronomic practices, and genomic markers. In common beans, they have discovered genes linked to heat tolerance and drought resistance, including potential adaptive genes. This strategy might be used to FIGS by developing a machine learning algorithm that combines environmental and genomic data. Thus, genebank accessions including passport data (ecogeographic and bio-geographic information) and genomic information (high throughput genotyping (GBS), next-generation sequencing, etc.) are employed in modelling FIGS to (i) mine specific adaptive traits and/or (ii) use environment-profiling to discover evolutionary hotspots. Further, trait-specific accessions are predicted using the model and confirmed through phenotyping (Sunitha et al., 2023) (Figure I.4).

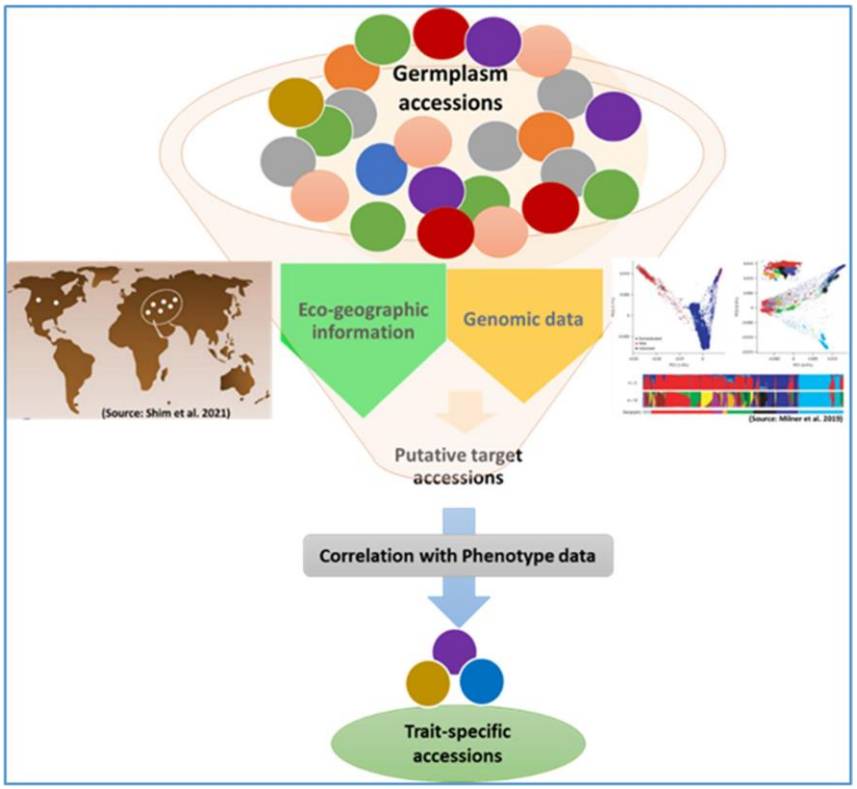


Figure I.4. Scheme for integration of genomic and eco-geographic information in focused identification of germplasm strategy (FIGS) (Sunitha et al., 2023)

The FIGS subset was successful in identifying new sources of resistance to the powdery mildew (Bhullar et al., 2010a; Vikas et al., 2020), Sunn pest (El Bouhssini et al., 2009), Russian wheat aphid (El Bouhssini et al., 2011), stem rust resistance (Bari et al., 2012) in wheat, drought tolerance in faba bean (Khazaei et al., 2013), net blotch in barley (Angessa & Li., 2016), barley

leaf rust (Amouzone et al., 2022). Therefore, the number of accessions for future study and for further analysis can be manageable by using these data mining approaches while maintaining good allelic variance in the subsets with high probability of finding the sought adaptive trait.

I.6.1 Genebank genomics: Unlocking the genetic potential of gene bank germplasm

Globally, gene banks are the major sources of genetically diverse material that contains genes and alleles that control useful traits, which are crucial for crop improvement through breeding (Tanksley & McCouch., 1997). The genetic gain in breeding programs for desirable traits including high yield and resistance to major abiotic and biotic stresses are supported by novel alleles discovered within gene bank genetic resources (González et al., 2018; Roa et al., 2016). More than 1750 genebanks around the world preserve a total of 7.4 million accessions (FAOSTAT., 2010). However, owing to the lack of evaluation for agronomic traits or to poor phenotypic and genotypic characterization, less than 10% of these accessions are employed by breeding programs (Patterson et al., 2006). Thus, gene banks need to move beyond providing basic passport data that defines only the identity and origin of the genetic resources, to thoroughly cataloguing and making publicly available additional information on accessions such as agronomic, physiological, and genetic traits that meets the specific needs of end-users, in order to improve the utilization of germplasm (Anglin et al., 2018; Díez et al., 2018). Insufficient genetic information about most of the holdings in gene banks makes plant breeders even more reluctant and McCouch et al in (2013) suggested a three steps strategy that combines genomics and phenomics with effective database management to maximize the use of genebanks holdings. The next-generation genome sequencing and the advancement of DNA technologies have increased the application of genomics by gene banks. Furthermore, recent studies in barley have demonstrated the abundance of single-nucleotide polymorphisms made possible by low-cost high-throughput genotyping which allowed to bridge the gaps between genetic resources conservation and their use in plant breeding (Milner et al., 2019).

I.7 The pathogen: *Rhynchosporium commune*

I.7.1 History and taxonomy

Scald was discovered by Oudemans in 1897 in the Netherlands on rye (*Secale cereale*) and he reported it under the name of *Marsonia secalis* n. sp. A detailed description of the disease is given by Frank in October 1897, having found it on rye and barley in Germany and he designated the fungus *Rhynchosporium graminicola* Heinsen. Brunner et al in (2007) presumed that Scandinavia is the predicted origin of the *R. commune.*

Davis in 1919 and 1921 and Caldwell in 1937 proposed the combination of the two names and accepted as *Rhynchosporium. secalis*. *R. commune* was given the *Rhynchosporium secalis*, while recently Crous et al., (2021) suggested renaming *R. commune* as *R. graminicola.*

In addition, Caldwell in 1937 isolated other *Rhynchosporium* species from *Dactylis glomerata* called *Rhynchosporium orthosporum* which is morphologically different from other species because of the typical beaked form of the conidia. The comparisons of ITS (internal transcribed spacer) and mating-type DNA sequences, confirmed that the pathogen is a member of the Helotiales, because the species is closely related to the helotialean plant pathogens *Pyrenopeziza brassicae* and *Oculimacula yallundae* (Goodwin., 2002; Saricaoglu., 2020). Zaffarano et al., (2011) reclassified *Rhynchosporium* into three species. Additionally, a recent study showed that *R. commune* has been detected and isolated from *Avena sativa*, *H. murinum* subsp. *glaucum,* and *Festuca perennis* (Seifollahi et al., 2018).

I.7.2 *Rhynchosporium commune* symptoms, biology and epidemiology

Scald lesions are easily identified and are typical oval-shaped areas or elongated spots. *Rhynchosporium commune* symptoms can be found on the coleoptiles, leaves, leaf sheaths, floral bracts, stem, glumes and awns (Hermannsson & Sverrisson., 2003; Mather et al., 1997). First symptoms appear on barley leaves as small dark or water-soaked, gray spots. Later they dry and bleach, becoming pale brown with a dark purple margin (Davis., 2003). When the disease develops the lesions enlarge and colease and could reduce photosynthetic area (Auriol et al., 1978) and could destroy the whole plant green leaf area leading to the death of the plant (Caldwell., 1937; Habgood & Hayes., 1971; Skoropad., 1959). Scald symptoms appear within 10-14 days under optimum conditions (Patil et al., 2002) (Figures I.4 and I.5).

Figure I.5. *R. commune* symptoms under Marchouch conditions at adult plant stage

Figure I.6. Resistant and highly susceptible reactions to *R. commune isolate* at the seedling stage

Rhynchosporium commune is a seed borne disease found in the endosperm and the seed coats and the infected seeds are responsible for the survival and spread of the disease (Ríos et al., 2007; Salamati et al., 2000). In addition, *R. commune* grows from the seed then spreads into roots and shoots and from there colonizes the plant. Several studies showed that for several months the seed-borne infection remains symptomless (Avrova & Knogge., 2012; Fountaine et al., 2010). In a study by Zhan et al in (2008) reported that the infection of *R. commune* via rain splash dispersal remains symptomless as the biotic phase does not produce the characteristic symptoms of scald shown in other phases. The fungus is unable to survive in the soil, however it could be spread by wind (Caldwell., 1937). Diseased volunteer barley and infected debris can be a primary source of inoculum and results in a severe scald epidemy (Shipton et al., 1974). Leaf blotch is a polycyclic disease and several generations of the pathogen occur before the development of the symptoms (Zhan et al., 2008a). Foster and Fitt in (2003) reported that the pathogen has a teleomorph closely related to *Pyrenopyziza brassicae* and *Oculimacula yallundae*. The sexual stage of the pathogen is still unknown (Abang et al., 2006).

The pathogen has a long incubation period, it appears and develops during cool, semi-humid and humid periods with wide range of temperature between 60°F to 68°F (Zhang et al., 2020). The conidia of *Rhynchosporium commune* are spread among plants by water-splash or air currents and can survive from season to season on barley stubble as dormant mycelium or

persists on grass or on plant debris for up to a year (Fitt et al., 2012). The amount of inoculum presents in leaves and in stubble debris influences the disease severity. Additionally, the time of the disease appearance determines the level of yield losses. If the disease appears and spreads late in the season, yield losses are low. In contrast, when scald appears and develops early in the season, it can cause serious damages. Epidemics of scald are seen under low temperatures (40°F to 75°F), frequent rainfall and dense crop canopies, which can maintain the humidity on the leaves. Hot and dry conditions are inhibitory of the disease progress (Zhang et al., 2020). The pathogen is able to colonize and sporulate on the resistant and susceptible barley varieties without causing visual symptoms, but can serve as a potential source of inoculum for later infections (Atkins et al., 2010; Avrova & Knogge., 2012; Zhan et al., 2008a).

I.7.3 Geographical distribution and economic importance

Rhynchosporium commune is a serious foliar disease spread around the world and found in all continents, but more frequent under cool and semi-humid barley growing regions (Zhan et al., 2008b).Yield loss can amount to more than 40% (Paulitz and Steffenson., 2011). Scald is a major biotic constraint to barley production in South Africa and in East Africa (Zaffarano et al., 2006), North America (Cromey., 2003; Turkington et al., 2003), causing severe damage in Tunisia, particularly in northern, northwestern and central parts of the country with 44% yield reduction in highly susceptible cultivars (Cherif et al., 1994; Yahyaoui., 2003; Bouajila et al., 2006). It has a significant negative impact on crop productivity in western Canada (Turkington et al., 2011), in Alberta (www.albertabarley.com) and in Germany particularly on winter barley (Kraatz., 2010 https://promedmail.org/post/20100428.1370). It can be severe in United States (Haskell., 1926; Caldwell., 1937; Riddle and Suneson., 1948; Riddle and Briggs., 1950; Schaller., 1951; Moseman., 1956; Webster et al., 1980). Severe scald epidemics are observed in Japan where most commercial cultivars are susceptible (Yamada and Shiomi., 1954; Arai., 1991; Aoki et al., 2011).

Scald is considered economically important in the southern, western and northern parts of Australia (Wallwork., 1996; Craddock., 2003; Zaffarano et al., 2006; Castleman., 2016). In Western Australia, yield losses ranged between 20 to 40% (Khan., 1986). It can cause losses of 50% or more in susceptible varieties under favorable conditions in southern of Australia (Wallwork and Gontar., 2015). In the state of Victoria, yield loss reached 45% (Brown., 1985) and in Trøndelag region in central Norway it ranged from 7 to 28 % (Salamati et al., 1997).

The disease is also reported in Britain (Anon., 1964; Jenkins & Jemmett., 1967) and the Soviet Union (Drapatyi., 1978). Scald was reported as the major disease for the southern Pampeana region of Argentina (Amieva et al., 1973; Carmona et al., 1997; Carmona & Barreto., 2003). In Türkiye, a survey on barley leaf diseases was conducted in 2019-2020 in 54 barley fields across 5 provinces in Central Anatolia, namely Ankara, Eskişehir, Kırıkkale, Kırşehir, and Yozgat. The surveys' results showed that scald was one of the most prevalent barley disease (Turgay et al., 2022). In addition, among the most destructive diseases found in 50 barley fields analyzed in the 2018 study conducted in the Bala area of Ankara was scald disease (Ertürk et al., 2018). Jenkins and Jemmett (1967) in United Kingdom reported yield losses of 35–40%. Franco in (2016) reported that scald still costing £10.8 million losses despite the use of fungicides (https://croprotect.com). In Northern Ireland, it is the most destructive and costly disease which can cause significant yield losses in both spring and winter barley (AFBI., 2012; https://cereals.ahdb.org.uk). At Lubeck in Germany, Craig and McLean in (2010) reported that up to 57% of leaf area is affected by scald with 0.7t/ha grain yield loss recorded. Several studies have documented yield losses of barley caused by scald can reach about 100% in countries where barley is continuously planted without crop rotation, including Morocco, Ethiopia, Tunisia, Peru, Colombia, Bolivia, Ecuador, Türkiye, Syria, and Iran (Abang et al., 2006; Arabi et al., 2008; Bouajila et al., 2010; Beigi et al., 2018). In Finland, scald was prevalent during all seasons, but only in 2015 was more severe scald reported in Denmark, Sweden, and Norway (Ajalli & Salehi., 2012). Further, In Ethiopia, scald is among widely distributed and destructive diseases in cool highland areas and yield losses reaching about 67% have been recorded (Abebe., 2021). Several million hectares of barley are affected, spanning across North Africa, West Asia, East Africa (Ethiopia and Eritrea), Yemen, Central Asia, the Andean nations (Bolivia, Ecuador, Peru, and Bolivia), and the Far East (Yahyaoui et al., 2004).

I.7.4 Host plant species

Several studies reported that *Rhynchosporium commune* is able to infect a broad range of plant species, nearly 70 species belonging to 20 genera**.** The pathogen was originally described from rye and was found in all regions where rye is cultivated (Goodwin et al., 1993; Ishkova et al., 2002; Zaffarano et al., 2006, 2011). Analyses of nucleotide sequences of NIP1, indicated that *R. secalis* started to colonize barley ~5000–7500 years after the domestication of barley (Brunner et al., 2007) that occurred ~10,000 B.P. (Badr et al., 2000). It is also infecting other species of *Hordeum* and *Agropyrum* (Zaffarano et al., 2008) and various species of wheat (*Triticum monococcum* L., *T. durum* Desf., *T. aestivum* L.) and triticale (× *Triticosecale* Wittm).

The wild grass species are the main hosts of *R. commune*, which can be an important reservoir of the most virulent strains to complete its sexual cycle (Linde et al., 2016; Seifollahi et al., 2018). Further, *R. commune* is able to invade *Hordeum* spp, *Bromus diandrus, H. murinum* subsp. *glaucum, Festuca perennis and Avena sativa* (Seifollahi et al., 2018; Zaffarano et al., 2011). The wide host adaptation of the fugus is governed by effector proteins, which prevent the pathogen from growing in plants and hence prolong the biotrophic phase (Clark et al., 2008; Penselin et al., 2016).

I.7.5 Control measure of scald disease

Control of scald disease is becoming hard and difficult, due to the high genetic diversity of the pathogen populations, which are able to change rapidly (Salamati et al., 2000) and also due to the spontaneous mutation (Williams et al., 2003a). Zaffaranno et al in (2006) detected a large diversity in *R. commune* populations using molecular markers with 60% of the world RFLP-based diversity found in a single barley field and about 40 % variation in 1 m^2 sampling area. Additionally, after a few cycles of asexual reproduction, new genotypes of *R. commune* were detected (Williams et al., 2003; Zhang et al., 2020). Other challenges facing the control of scald disease are its capacity to survive, colonize and sporulate on the resistant varieties which can be a source of inoculum for further infection (Atkins et al., 2010). Many studies using PCR detected symptomless presence of *Rhynchosporium* on the seeds (Lee et al., 2002), which allowed the transmission of the pathogen from the seed to seedling (Fountaine et al., 2005). Seed-borne plays a significant role in the rapid spread of the disease and the survival of the fungus (Salamati et al., 2000).

Using fungicides and cultural practices have not proved to be effective and sustainable strategies for the management and control of scald disease (Zhang et al., 2020). Resistance of *R. commune* populations to multiple fungicides has been reported by several studies. *Rhychosporium* increasing resistance to epoxiconazole and flusilazole fungicides (Oxley et al., 2003). Under widespread and continuous use of benzimidazole fungicides, resistant isolates of *R. secalis* become rapidly predominant and spread to neighboring populations (Taggart et al., 1999). In addition, new strains were developed that are surviving the treatments by the frequently used fungicides (Avrova and Knogge., 2012). However, various studies showed that an unexposed *Rhynchosporium* populations to epoxiconazole, tebuconazole and flusilazole fungicides are ten times more sensitive than exposed population (Robbertste et al., 2002; Cooke et al., 2004). Genetic resistance remains the most economic and environmentally-friendly way

to control scald disease, but become ineffective after several widespread commercial use (Oxley et al., 2003). Xi et al in (2002) have reported that R-genes in commercial cultivars had a short effective life. However, breeders look for more durable resistance and race-non-specific for effective control of scald such as partial resistance (Parlevliet., 1975). Interestingly, introgression and pyramiding multiple different resistance loci including major and minor genes for stable and more durable scald resistance is needed for breeding program (Zhang et al., 2020). The most sustainable and effective strategies for management of scald disease needs improved understanding of *R. commune* populations biology and its interactions with host and should integrate resistance gene deployment and other strategies such as crop rotations for decreasing the amount of primary inoculum and bury the infected debris after harvest (Shipton et al., 1974). Decreasing nitrogen applications, sowing and canopy density during growing season can decrease severity of scald epidemic (Hoad & Wilson., 2006). Newton et al in (2004) have reported that plant habits can affect the severity of scald, being less severe on plants with the ari-eGP dwarfing gene associated with erect juvenile habit than on those with the sdw1dwarfing gene associated with the semi-prostrate juvenile leaf habit. Decreased rate of inoculum occurs through wind-borne ascospore by using mixture of resistant and susceptible cultivars (McDonald et al., 1988).

The use of highly heterogeneous cultivar (Newton et al., 1997), achievement a geometry of spatial deployment of resistant cultivars through mixing in farm seed drills play a key role for the control the disease (Newton., 2007). On commercial barley varieties, a single approach will not provide effective and sustainable control of scald epidemics. The management and control of the disease needs more knowledge in breeding program about mechanisms of minor and complete resistance, understanding changing from asymptomatic infection of *Rhynchosporium* to visual symptom expression and the factors and of the causes of symptomless infection. However, visual symptom expression does not provide a reliable assessment of resistance to scald and will need the use of quantitative PCR (Fountaine., 2005).

I.7.6 Population genetics of *Rhynchosporium commune*

Studying and understanding the genetic diversity of the pathogen population is crucial for improvement of disease resistance in future global barley breeding efforts. Various evolutionary forces such as random genetic drift, mutation, mating systems and genotype flow alter the pathogen population. The pathogens possess a high level of mutation rates, genotype flow, large effective population sizes, and multiple reproduction methods can easily breakdown the

resistant plant compared to the pathogens that have a low level of mutation rate, less potential for gene flow, small population sizes, and stringent asexual reproduction (Kaur et al., 2021).

The genetic structure studies of the pathogen population have demonstrated that *R. commune* population can maintain a high level of genetic diversity at a microscale (Linde et al., 2009a; McDonald., 2015). Several studies have revealed a high virulence diversity in *R. commune* populations worldwide, which provides further evidence that the pathogen population is characterized by its high genetic and phenotypic diversity (Figure I.6) (Bouajila et al., 2010; Stefansson et al., 2012, 2014). Zaffarano et al in (2006) found that the genetic diversity at a single geographic location was more than 70% of the total genetic variation in a region within Europe. While, another study of *R. commune* isolates demonstrated that 19% of gene diversity was distributed among sampling sites within fields, and 76% of gene diversity was distributed within the sampling site area of approximately 1 m^2 while only 5% of gene diversity was distributed among fields (McDonald et al., 1999a). Further, Mcdonald in (2015) reported that Scandinavia contains the greatest virulence diversity of *R. commune.*

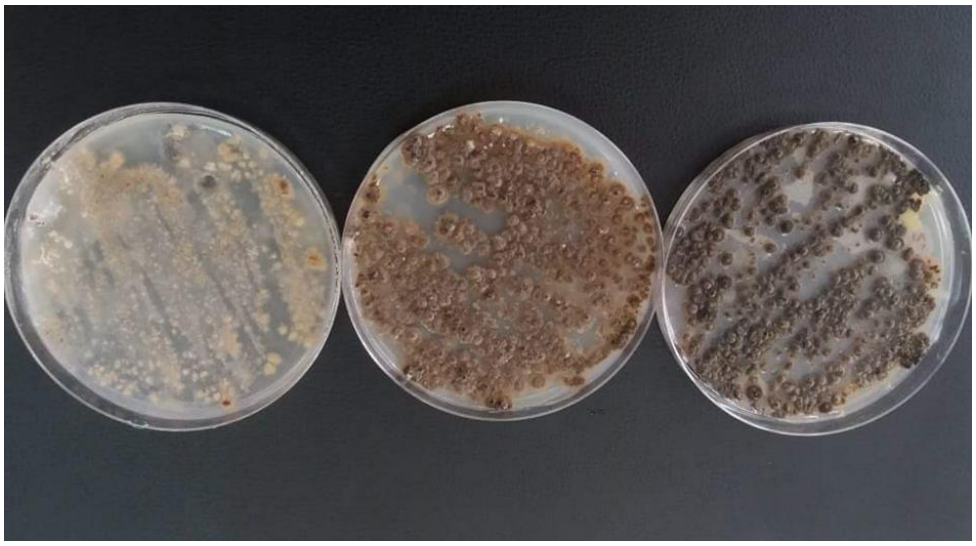


Figure I.7. Diversity of *R. commune* isolates colonies

I.7.7 Host–pathogen interactions

The most significant and widespread aspect of plant disease resistance is gene for gene interactions, also known as active plant defense (APD) which was discovered by Flor in (1942). Gene for gene interaction sets a milestone in understanding the biochemical and genetic basis of plant diseases and several components involved in plant–pathogen interactions. Several studies by Flor (1971) showed that the presence of dominant (*R*) genes in different cultivars conferred resistance to specific strains of flax rust. In this concept, host defense responses are

activated by the recognition of a pathogen avirulence (Avr) gene by a cognate resistance (R) gene in the host. In addition, gene-for-gene relationships helped in the identification of several R genes in the host and their corresponding Avr genes in the pathogen.

Gene-for-gene interactions were found between hosts and many other pathogens and pests, including fungi, bacteria, viruses, insects and nematodes (Martin et al., 2003). The genetic analyzes of the plants and pathogens revealed that the inheritance of both resistance in the host and parasite ability to cause disease is controlled by pairs of matching genes. Robinson and Raoul (1987) demonstrated that the plants produce a specific *R* gene that are resistant toward a pathogen which produce corresponding *Avr* gene. In addition, recent advances in bioinformatics, genomics and genetics are providing greater prospects for deeper understanding of host-pathogen interaction (Kaur et al., 2021).

- **The biochemical basis for gene for gene interactions**

Several models have been developed to explain the molecular and biochemical basis of the gene-for-gene interactions, based on studying and understanding of several Avr genes and R resistant genes in different crops (Kaur et al., 2021). These include ion channel defense model (Gabriel et al., 1988), dimer model (Ellingboe., 1982), and elicitor-receptor model (Keen and Bruegger., 1977). The elicitor-receptor model states that the avirulence gene (Avr) encoding elicitor protein can react directly or indirectly with the receptor molecule produced by R gene of the host plant (Agrios., 1969; Biezen and Jones., 1998). The elicitors/Avr genes products can be general elicitors and race-specific. There are two ways by which gene for gene interactions can be determined:

- Compatible relationship: the susceptible response is a result of the specific molecules resulting from the interactions between the virulence alleles (Avr) in the pathogen and the susceptibility alleles (r) in the plant.
- Incompatible relationship: The alleles for resistance (R) in the host and those for avirulence (Avr) in the pathogen produce molecules, which specifically recognize each other and produce resistance response in the host.

- **Molecular basis for gene-for-gene interactions**

The resistant and susceptible reaction of the host is depended on the binding of elicitor-receptor protein, basically the protein product of the avirulence gene/ elicitor can be recognized by its complement gene in the host/ receptor and vice versa.

Genetically, there are four combinations of Avr and R genes:

a) First gene combination (AR): the elicitor molecules encoded by the pathogen Avr gene was recognized by the R gene-encoded host receptor and activates the defense reaction against the pathogen which make the host resistant.

b) Second gene combination (Ar): the R gene-encoded host receptor cannot recognize the elicitor molecules encoded by the pathogen Avr gene or more precisely the host lacks the receptors for the pathogen receptors and nonhost defense is triggered which rendering the host susceptible.

c) Third gene combination (aR): the R gene-encoded host receptor cannot recognize the elicitor molecules encoded by the pathogen Avr gene, hence the host was not able to activate the defense reaction and virulence gene operates which make the host susceptible.

d) Fourth gene combination (ar): nonhost defense reaction is triggered making host susceptible as neither the pathogen elicitor is created nor the host receptor exists.

Genetically, "Central Dogma of Plant Pathology" model was established to elucidate the coevolution of both the R and Avr genes or between plants and pathogen, which contains four steps. The first step of this model named PAMP triggered immunity (PTI), which is the defense reaction generated in response to the sensing of PAMPs. Primarily, the pathogen was able to recognize a specific host, hence the host sense various structures or molecules which could be a part or the pathogen product (chitin, flagellin) known as pathogen associated molecular patterns (PAMPs also called MAMPs) and trigger the defense mechanism. In the second step, the pathogenic bacteria have evolved their type three secretion system (TTSS) to convey proteins and suppress the basal immunity, PTI and permitting potential accumulation of bacteria in the plant apoplast. The plant has evolved other receptors or plant resistance proteins, such as a TIR-NB-LRR protein, which can detect the effector activities and restore the resistance by effector triggered immunity (ETI). Similarly, the pathogen evolved to survive by ignoring and avoiding the R gene-mediated defense by altering the effectors that trigger those responses (Bent and Mackey., 2007).

The R gene-encoded host receptor identifies the elicitor molecules encoded by the pathogen Avr gene and activates defense reaction. Every generation of the *R. commune* life cycle, the gene-for-gene interactions occur between the disease resistance genes (R genes) in the plant and the corresponding avirulence effectors in the *R. commune* (Barua et al., 1993). Wevelsiep et al in (1993) identified three necrosis inducing peptides, necrosis inducing peptide1, necrosis

inducing peptide2, and necrosis inducing peptide3 (NIP1, NIP2, and NIP3) which are important during the thin hyphal forming stage in *R. commune.* The NIP2 and NIP3 are the most important proteins for the *R. commune* populations, which were found in diverse isolates of the pathogen populations (Schurch et al., 2004; Stefansson et al., 2014). A study of the expression analysis revealed that NIP2 and NIP3 transcripts are synthesized after inoculation of host plants, while the NIP1 transcripts are present in spores (Kirsten et al., 2012). At the early growth stages of infection, when fungal hyphae spread before the growth of dense subcuticular stroma, the three proteins are functionally very important (Schurch et al., 2004). Most of the *R. commune* populations possess NIP2 and NIP3 proteins, while up to 45% of NIP1 showing deletion frequency.

Two distinct NIP1 paralogs were detected in almost all *Rhynchosporium* species, designated as NIP1A and NIP1B. Across *Rhynchosporium* species, variation in NIP1A and NIP1B copy number was reported (Mohd-Assaad et al., 2019). A significant positive selection was reported in the NIP1A paralog, indicating that this paralog is the dominant effector that is co-evolving with host receptors. Further, the most significant influence on virulence of the pathogen population was detected in the NIP1A (Mohd-Assaad et al., 2019). Seven to ten members of NIP2 families were identified in *R. commune* isolates (Penselin et al., 2016). The NIP1, NIP2, and NIP3 proteins are functionally important at the early growth stages of infection, when fungal hyphae spread before the growth of dense subcuticular stroma.

The fungal biomass has an effect on the production of the three proteins, when it is increased the production of these proteins dramatically decreases, leading to the early influence of these proteins on fungal virulence (Schurch et al., 2004). The protein NIP1 has two functions, it can play a role of an effector and an elicitor (Wevelsiep et al., 1993), while the NIP2 and NIP3 had no function as elicitors (Hahn et al., 1993). Several studies have demonstrated that *R. commune* strains possessing nonfunctional or missing NIP1 showed lower virulence than the strains carrying a functional NIP1 gene (Stefansson et al., 2014; Mohd-Assaad et al., 2019). Furthermore, study by Stukenbrock and McDonald in (2009) have showed that the *R. commune* population with mutated NIP1 or lacking this protein can overcome cultivars possessing Rrs1.

The NIP1 protein is the product of the avirulence gene AvrRrs1 (Rohe Ket al., 1995), and is able to induce the reactions of the Rrs1 gene and facilitate leaf necrosis in barley (Hahn et al.,1993). Furthermore, Steiner-Lange et al in (2003) have demonstrated that NIP1 protein is able to induce the expression of pathogenesis-related ten gene in leaves of Rrs1 barley plants. Studies demonstrated that the NIP1 is able to bind to plasma membranes of (Rrs1) and

susceptible (rrs1) barley genotypes and promote the activity of barley H+-ATPase (Wevelsiep et al., 1993; van Slot et al., 2007).

I.7.8 Genetic resistance to *Rynchosporium commune*

Genetically, the plant disease resistance can be classified into two classes based on the level and the strength of resistance: complete resistance, also known as vertical resistance, major gene, qualitative resistance or race specific is mediated by one or two resistance genes and follow Flor's gene-for-gene interaction, and the second type of disease resistance is partial resistance also termed as horizontal, minor gene, quantitative or race nonspecific resistance is governed by multiple genes or quantitative trait loci (QTLs) (Kou and Wang., 2010; Zhang and Wang., 2013).

The plants are continuously invaded by various pathogens, to overcome this invasion the plant have involved two tiers of immune receptors. The host detects the microbial signature by cell surface-resident pattern recognition receptors (PRRs) and recognizes the pathogen effectors by intracellular nucleotide binding domain leucine-rich repeat (NLR) proteins (Mang et al., 2017). In general, the molecular mechanisms of plant resistance against the pathogens are elucidating with two-tiered innate immune system. The first branch of plant immunity, named pattern-triggered immunity (PTI), or plant-derived damage associated molecular or basal resistance and the second branch of plant immunity is called effector triggered immunity (ETI) or gene-for-gene resistance (Jones and Dangl., 2006; Thomma et al., 2011; Monaghan and Zipfel., 2012).The pattern-triggered immunity (PTI) is activated by microbe-associated molecular patterns (MAMPs) that are recognized by plasma membrane-localized pattern recognition receptors (PRRs), which are receptor-kinase proteins or receptor-like proteins (Boller and Felix., 2009; Couto and Zipfel., 2016; Yu et al., 2017), and the ETI is triggered upon detection of of pathogen effectors via intracellular immune receptors, which are often encoded by nucleotide binding domain leucine-rich repeat (NLR or NB-LRR) proteins (Jones and Dangl., 2006; Jones et al., 2016; Maekawa et al., 2011).

In general, the first branch of plant immunity (PTI) is quantitative resistance, and the second mechanism (ETI) is qualitative resistance in many plant–pathogen pathosystems (Zhang and Wang., 2013).

I.7.8.1 Major and minor genes for scald disease resistance

Resistance to *Rhynchosporium commune* in barley is mediated by both major genes or host-specific genes "complete resistance" and minor genes "partial resistance", thus the resistance

can be mediated by an ETI or a PTI mechanisms (Zhan et al., 2008). Durable disease control requires a combination of both types of resistance into new cultivars (Walters et al., 2012). Relatively few resistance loci against *R. commune* have been discovered that can be employed in breeding programs, compared to other barley diseases (Dracatos et al., 2019; Clare et al., 2020; Zhang et al., 2020). Major genes have been frequently detected using specific strains at the seedling stage and provide at all growth stages a high level of resistance (Zhan et al., 2008). However, the minor gene resistance is often detected at the adult plant stage and provide a partial level of resistance (Wallwork and Grcic., 2011; Zhan et al., 2008).

The first study on the inheritance of scald resistance was in 1929 by Mackie, identified that resistance in an unspecified barley variety was governed by a single recessive gene. Several qualitative and quantitative resistance genes against *R. secalis* have been identified and mapped. There are various sources of resistance derived from *Hordeum vulgare* ssp. *vulgare* (Dyck and Schaller., 1961; Habgood and Hayes., 1971; Schweizer et al. 1995, 2004; Graner and Tekauz., 1996; Cheong et al., 2006; Wagner et al., 2008; Silvar et al., 2010, Hofmann et al., 2013), from wild barley *Hordeum vulgare* ssp. *spontaneum* (Garvin et al., 2000; Genger et al., 2003; von Korff et al., 2005; Yun et al., 2005) and from *Hordeum bulbosum* (Pickering et al., 2006). Bryner in (1957) was the first to use the designation for genes controlling resistance to *R. secalis* and assigned the symbol Rhar to a dominant gene identified in Brier. Bjørnstadt et al in (2002) introduced a new classification using Rrs prefix which follow the Rrs nomenclature for Reaction/Resistance to *Rhyncosporium secalis.* Due to the nomenclature alterations of the *R. commune* resistant loci, the numbering of Rrs loci is not strictly sequential.

Across the barley genome, several QTLs and genes to *R. commune* resistance have been reported. To date, limited number of R genes (11 major Rrs genes) including *Rrs1, Rrs2*, Rrs3, *Rrs4, Rrs12, Rrs13, Rrs14, Rrs15, Rrs16, Rrs17* (*Rrs15* (CI8288)), and *Rrs18* controlling resistance to *R. commune* have been identified (Zhang et al., 2020). The rationale behind the relative lack of major gene identification is the limited genetic diversity among the barley germplasm caused by the multiple reselections of the same allele combinations from different cultivars (Williams et al., 2003).

The first locus discovered and mapped on chromosome 3H was Rrs1 with many alleles, formerly known as the Rh-Rh3-Rh4 locus (Thomas et al., 1995; Graner and Tekauz., 1996; Williams et al., 2001; Grønnerød et al., 2002; Bjørnstad et al., 2002; Genger et al., 2003; Bjornstad et al., 2002; Read et al., 2003; Genger et al., 2005; Hofmann et al., 2013). A number of molecular markers are available for this locus (Graner and Tekauz 1996; Penner et al. 1996;

Russell et al., 1997; Grønnerød et al., 2002). The main feature of the Rrs1 has been mapped as both major and minor genes (qualitative and quantitative genes) (Li and Zhou., 2011; Wagner et al., 2008; Looseley et al., 2014; von Korff et al., 2005; Shtaya et al., 2006). Thus, Rrs1 is a complex locus found at the centromeric region of chromosome 3H (Büttner et al., 2020; Zhang et al., 2020).

At the resistant Rrs1 locus, there are 27 major or minor QTLs controlling scald disease resistance, which are spreading from 429 to 503 Mb Mb on version 2 of the Morex genome assembly and have been detected under both controlled conditions using specific isolates and under field conditions (Zhang et al., 2020). Further, two loci conferring resistant to scald disease have been detected at 383.9 and 573.5 Mb and designated as Rrs1 (Genger et al., 2003; Zhang et al., 2020). About 22 major QTLs were detected at the seedling stage using specific isolates and only five QTLs were identified at adult plant stage (Spaner et al., 1998; Williams et al., 2001; Grønnerød et al., 2002; Jensen et al., 2002; Sayed et al., 2004; Shtaya et al., 2006; Li and Zhou., 2011). Further, Patil et al in (2003) studied the inheritance of resistance against isolate 404 of scald and has mapped another resistance gene, named $Rrs4_{CI11549}$ at 22 cM distal to Rrs1 on chromosome 3HL using HVM60 molecular marker.

Adding to the complexity, several resistance QTLs are located in the same region on the chromosome 3H as Rrs1 locus. However, there is a lack of clarity if these QTLs are part of a closely linked gene cluster or are alleles of the same gene (Patil et al., 2002). A recent study by Looseley et al in (2020), showed that about 25 cultivars from UK possess the resistant Rrs1 (Rh4 type) locus, and indicated the presence of relatively few independent introgressions of the Rrs1 (Rh4 type) locus in UK genotypes, and also postulated that the resistance of this locus is governed by a presence/absence variant that is absent in the current Morex genome sequence. Only the Rrs1 locus has been detected to have a corresponding avirulence protein, necrosis inducing protein 1 (NIP1), against scald (Rohe et al., 1995). *R. commune* strains with an avirulent allele of NIP1, Rrs1 prevents leaf cuticle penetration and subcuticular development (Carisse et al., 2000; Lehnackers and Knogge., 1990; Thirugnanasambandam et al., 2011).

In the literature, there is an uncertainty about the nomenclature of the two loci which are termed as Rrs15. On the chromosome 2HS, Schweizer set al in (2004) detected a single dominant gene $Rrs15_{CI8288,}$ using STS marker GemS13 developed from S13M52, and the name of QTL Rrs17 (Rrs15 (CI8288)) locus was retained by Wagner et al in (2008), which is mapped at 10.4 Mb and was detected under both adult plant and seedling stages. In addition, Zhan et al in (2008) renamed Rrs15 (CI8288) locus as Rrs17 to distinguish it from the locus on 7H. Another study

by Cheong et al in (2006) identified under field conditions a major QTL that overlaps with the Rrs15 genome position.

On the long arm of barley chromosome 7H, Rrs15 locus was detected controlling resistance to scald, derived from wild barley *H. spontaneum* and has been mapped at 626.3 Mb by Genger et al (2005). A major QTL Rrs14 was identified on the chromosome 1H, and mapped at 2.8 Mb (Garvin et al., 2000). Further, another QTL (Rrs-1H-1-4) originated from *Hordeum spontaneum* was detected close to Rrs14 (Yun et al., 2005). A dominant gene for scald resistance Rrs16, originated from *Hordeum bulbosum* has been mapped on 4HS by Pickering et al. (2006). Two different studies have detected six QTLs controlling scald disease closely mapped to Rrs16 (Wallwork et al., 2014; Wang et al., 2014).

The Rrs13 gene was detected on the short arm of chromosome 6H and sourced from an interspecific cross with *H. spontaneum*. Two markers are linked to this gene, Cxp3 and MWG916 (Abbott et al., 1995; Genger et al., 2003b). Furthermore, QTLs for scald resistance at adult plant stage were also identified at the Rrs13 locus (Spaner et al., 1998; Shtaya et al., 2006). On chromosome 6H, new locus named Rrs18 was detected and has been located at 11.8 Mb by using pseudomolecules Morex v. 2.0 2019 (Coulter et al., 2019). After projecting Rrs13 and Rrs18 onto the barley physical map, the distance between the two loci was approximately 10 Mb (Coulter et al., 2019). There has been a further new major QTL named Qsc2.6H and was detected in the same region as Rrs18 (10.9–12.0 Mb) by Zantinge et al in (2019).

A number of resistant QTLs have been identified on chromosome 7H. Dyck and Schaller (1961) identified Rrs2 locus (formerly called Rh2) and was mapped by Schweizer et al in (1995) on the distal part of the short arm of chromosome 7H at an interval of 0.08 cM between markers 693M6_6 and P1D23R (Hanemann et al., 2009). The Rrs2 locus presented an important source of resistance for barley breeding program worldwide due to the effective resistance against most of *R. commune* isolates (Hanemann et al., 2009). Another resistant locus on chromosome 7H, termed Rrs12, originated from wild barley *H. spontaneum* has been mapped at 16.0 Mb (Abbott et al., 1992). In addition, different studies have reported the presence of additional nine different QTLs overlapping between Rrs2 and Rrs12 (Bjornstad et al., 2004; Cheong et al., 2006; Wagner et al., 2008; Wallwork et al., 2014). Across the barley genome, several minor QTLs for scald resistance have been located on chromosome 5H, while no major genes conferring scald disease resistance have been detected on this chromosome (Looseley et al., 2012; Coulter et al., 2019; Zantinge et al., 2019).

Therefore, novel sources of resistance to *R. commune* represent a valuable resource for plant breeders and particularly, quantitative resistance loci which have previously shown to be more

durable than major resistance loci in other host pathogen systems (Brun et al., 2010). Quantitative or partial resistance is assumed to be more durable, because the selection pressure is lower and the resistance is more difficult for the fungus to overcome. Several studies demonstrated that partial resistance could reduce scald disease severity (Williams and Owen., 1975; Kari and Grifths., 1993; Looseley et al., 2012). So far, over 150 QTLs conferring resistance to *R. commune* have been identified using biparental mapping populations and are found across all seven barley chromosomes (Büttner et al., 2020; Zhang et al., 2020; Cope et al., 2021; Hautsalo et al., 2021; Shaun James Clare et al., 2022). All QTLs detected in different studies with the molecular markers information and their positions in barley genome are presented in (Annex I.1.1 and Figure I.8)

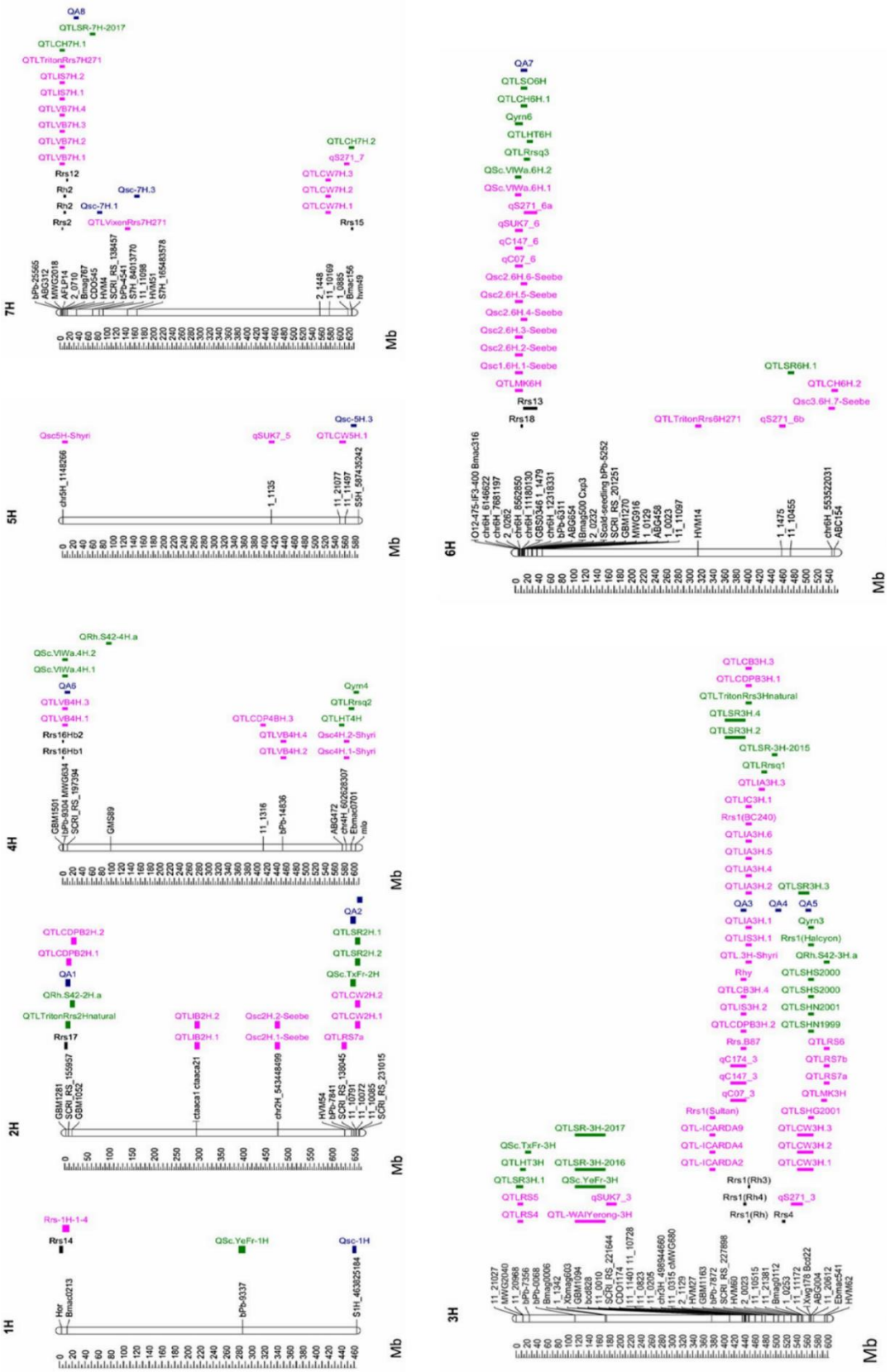


Figure I.8. Genetic map of SNP markers associated to scald disease resistance in barley (Zhang et al., 2020).

I.7.9 Candidate genes for *R. commune* resistance loci

Understanding the genetic basis of scald disease resistance in barley by identifying the genetic loci and the candidate genes is useful for developing new varieties. The BLAST searches are the widely used approach for searching for candidate genes of major scald resistance QTLs (Hanemann et al., 2009; Hofmann et al., 2013; Coulter et al., 2019). The candidate genes, their annotations and locations were found by subjecting the sequences of significant SNP markers to the BARLEYMAP (Cantalapiedra et al., 2015) or to the IPK barley server https://webblast.ipk-gatersleben.de/barley_ibsc/. To date, candidate genes (CG) have been published for only three Rrs genes. For the resistant loci Rrs1(Rh4 type), Rrs18, and QSc.VR4 receptor-like kinases were proposed as the causal genes (Looseley et al., 2020; coulter et al., 2019; Wang et al., 2020). Whereas, a cluster of Pectin Esterase Inhibitor genes (PEI) triggers the Rrs2 resistance response (Hanemann et al., 2009; Marzin et al., 2016).

In general, the actual candidate gene at the Rrs1 (Rh4 type) locus is still not detectable, due to the absence of this locus from the reference genome sequence of Morex (Mascher et al., 2017). Similarly, Marzin et al in (2016) indicated that due to the formation of the reference genome sequence from the susceptible cultivar Morex, the scald resistance gene Rrs2 locus could not be detected (Coulter et al., 2019). Ten high-confidence annotated genes have been detected at this resistant locus (Looseley et al., 2020). Further, Looseley et al in (2020) have developed a diagnostic marker based on single nucleotide polymorphisms (SNPs) for fine mapped of Rrs1 (Rh4 type) while a specific SNP was developed for the resistant Rrs2 locus by Hanemann et al in (2009). A study by Marzin et al (2016), of a family of putative pectin esterase inhibitor (PEI) genes at the Rrs2 locus, demonstrated that Rrs2 gene could be some combination of PEI genes or probably another known or unknown PEI gene.

Identification of genes location coding disease resistance could allow further manipulation and functional analysis of these genes, such as genes cloning. Nucleotide-binding site leucine-rich repeat (NBS–LRR) proteins or wall-associated kinases, were the first proteins detected of classically defined disease-resistance genes (*R* genes) and mediate pathogen recognition (Whitham et al., 1994; Mindrinos et al., 1994; Ade., 2006). In the last decade, most plant disease resistance genes that have been cloned encode NBS-LRR proteins (McHale et al., 2006; Dangl et al., 2013; Kourelis and van der Hoorn., 2018). To date, there has been at least one attempt to clone Rrs1 by Oldach in (2012), however none of the major scald resistance genes have been cloned. MutRenSeq and whole-exome capture cloning methods cannot detect the missed genes that are not present in the reference genome. Interestingly, to overcome these obstacles, the

development of a new gene cloning method called MutChromSeqdoe, which doesn't require fine mapping or the construction of a physical reference sequence across a map interval (Sánchez-Martín et al., 2016).

Genome size and LD decay (the rate at which LD declines with genetic or physical distance) influence a population's map resolution (i.e., the number of markers and density). It has been demonstrated that the rate at which (LD) decays across distance (genetic/physical) varies greatly and significantly among species, throughout the genome, and for loci within a population. The rapid decay of LD needed a large number of markers to be employed in the whole-genome association analysis (Semagn., 2010). By dividing the genome size by the distance at which LD decays, breeders can determine the number of markers required for GWAS using the rate of LD decay (Fedoruk et al., 2013).

I.8 Molecular markers in plant breeding and genetic resources conservation and use

The study of the genetic composition and variability within and among plant populations are, crucial for effective use and conservation of plant genetic resources, which is influenced by factors like mating systems, evolutionary history, and gene flow (Thoungamba., 2007).

Conventional methods, which depend on morphological traits, and cytological features, have been instrumental in understanding levels and patterns of variation, but had limitations, including environmental effects on trait expression (Avis., 2004; Nongdam., 2007). In contrast, recent advances in molecular marker technologies, particularly PCR-based markers, have significantly improved the accuracy of assessing genetic diversity (Andersen and Lübberstedt., 2003; Horst and Wenzel., 2005). Molecular markers, like SNPs (single nucleotide polymorphisms), AFLPs (amplified fragment length polymorphism), and SSRs (simple sequence repeats), enable precise genetic analysis, which useful in genebank management, genetic mapping, and marker-assisted selection. SNP markers, notably abundant and cost-effective, have become increasingly popular, leading to specialized genotyping arrays for various crops, transforming the fields of genetic resource conservation and plant breeding (Collard et al., 2005; Jiang., 2013; Nadeem et al., 2018; Reddy et al., 2021).

I.8.1 Advancement of next generation sequencing technology

Genomic research has seen remarkable advancements over the years, particularly in the area of DNA sequencing technologies (Kim C et al., 2016). The field of genomic, initially reliant on Sanger sequencing, transitioned to Next-Generation Sequencing (NGS) to overcome its limitations, including low throughput and high costs (E. Mardis., 2013). This shift, notably around the years between 2004 and 2006 which marked by the introduction of the 454 sequencing technology by Roche Applied Science. This innovative technology, enable to providing efficient, cost-effective, and high-throughput DNA sequencing. The 454 platform has the ability to generate a substantial amount of sequence data in relatively short runs. This platform was capable of producing 80-120 megabases (Mb) of DNA sequence data in read lengths of 200-300 base pairs (bp) within just 4 hours of operation. This was a massive improvement compared to Sanger sequencing, which required significantly more time and resources to generate a fraction of the data (Amom and Nongdam., 2017).

High-throughput genotyping techniques in barley genetics have significantly contributed and to advance the understanding of its genome since 2006. Innovations began with the Illumina GoldenGate assays, introducing 1,572 SNP markers (Fan et al., 2003; Close et al., 2009). Progress continued with the 9k Illumina Infinium iSelect Custom Genotyping BeadChip in 2009, increasing SNP markers to 9,842, enhancing genotypic resolution and accuracy (Comadran et al., 2012). Furthermore, iSelect technology evolved to analyze up to 700,000 targets per array, marking a new exploration era in barley genetics, further propelled by a detailed 5.1 Gbp genome assembly and chromosome-scale annotations for precise marker position (Beier et al., 2017; Mascher et al., 2017; Micha and Bayer., 2017).

I.9 Applications of advanced genomic tools and computer algorithms for scald resistance

I.9.1 Genomic tool: Genome wide association studies (GWAS)

In the past, linkage analysis of biparental mapping populations have been used to identify QTLs. More than 150 QTLs for scald resistance were identified using biparental mapping populations. Other robust approaches were used to detect new sources of resistance against *R. commune* population including genome-wide association studies (GWAS) and genomic selection.

Genome-wide association study (GWAS) is a powerful approach to map regions of the genome associated with traits across heterogeneous individuals and it is an efficient and effective tool to calculate the association between each marker and a phenotype of interest that has been evaluated across a diverse collection (Huang et al., 2014). High-resolution mapping can also be attributed to historical recombination events and the higher allele numbers that are integrated

in GWAS (Rafalski et al., 2010). The diversified maize (*Zea mays L.)* population and *Arabidopsis* were the first plants on which GWAS approach was utilized and reported (Tenaillon et al., 2001; Nordborg et al., 2002). Subsequently, the approach was applied to other crops, and a number of studies were reported (Rafalski et al., 2010). However, this strategy was first used in barley ten years ago (Caldwell et al., 2006; Stracke et al., 2007). The approach can identify causal loci underlying natural phenotypic variation by screening distantly related and heterogeneous individuals of a diverse collection with an ample density of genetic markers.

I.9.1.1 Scald resistance markers detected using GWAS

To date, three GWAS analysis for scald resistance have been reported and allowed to identify about 13 QTLs at adult plant stage under natural field conditions (Table 2 and Figure 2) (Gawenda et al., 2015; Looseley*et* al., 2018; Daba et al., 2019). The first study on GWAS analysis for *R. commune* reported by Gawenda et al., (2015) detected QTLG2H on chromosome 2H. Looseley et al in (2018), evaluated European spring barley germplasm under natural field conditions in Europe and indicated that the most significant QTL was Rrs1 on 3H. Other novel QTLs for *R. commune* resistance were detected on chromosomes 1H, 5H, and 7H using barley genotypes from Ethiopia, ICARDA and USA (Daba et al., 2019). The GWAS analysis of the three studies demonstrated that the genetic architecture of resistance against *R. commune* population is more complicated and the effects of resistance genes differ depending on environmental effects.

Table I.2. Summary of the scald resistance quantitative trait loci (QTL) from genome-wide association studies ordered by chromosome position giving logarithm of the odds (LOD) scores, percentage of phenotypic variation

QTL	Chr	Physical position (Mb)	LOD	Marker	Reference
Qsc-1H	1H	463.8	4.4	S1H_463825184	Daba et al. (2019)
QA1	2H	16.5	3.8	SCRI_RS_155957	Looseley et al. (2018)
QA2	2H	656.6	3.8	SCRI_RS_138045	Looseley et al. (2018)
QTLG2H	2H	669.2	-	SCRI_RS_231015	Gawenda et al. (2015)
QA3	3H	446.9	9.9	SCRI_RS_221644	Looseley et al. (2018)
QA4	3H	512.2	4.2	SCRI_RS_227898	Looseley et al. (2018)
QA5	3H	570.9	3.2	SCRI_RS_138723	Looseley et al. (2018)
QA6	4H	10.1	3.3	SCRI_RS_197394	Looseley et al. (2018)
Qsc-5H.3	5H	587.4	4.9	S5H_587435242	Daba et al. (2019)
QA7	6H	16.4	3.4	SCRI_RS_201251	Looseley et al. (2018)
QA8	7H	35.5	3.8	SCRI_RS_138457	Looseley et al. (2018)
Qsc-7H.1	7H	84.0	6.7	S7H_84013770	Daba et al. (2019)
Qsc-7H.3	7H	165.6	5.6	S7H_165483578	Daba et al. (2019)

I.9.2 Technological tool: Integrating artificial intelligence for enhanced crop improvement strategies

I.9.2.1 Machine learning algorithms

The overall objective of breeding program is accelerating genetic gain and shortening breeding cycle. Thus, with the advanced technology in computer science, plant breeding can become more accurate, robust, and capable of evaluating a wider set of variables. Machine learning (ML) is one of the most popular fields of Artificial Intelligence that enables computers

to learn from data, to identify appropriate patterns used to make decisions or predictions (Libbrecht and Noble., 2015).

The integration of AI technologies, including Machine Learning (ML), robotics, and computer vision, into plant breeding bring precision, and efficiency to various aspects of the breeding process, enabling researchers to select superior plant varieties with desired traits (Chandra et al., 2020). It is also leading to accelerated crop improvement and increased resilience against various challenges (Yoosefzadeh-Najafabadi et al., 2021). However, ML faces several challenges, including those pertaining to the data requirements and computational resources needed to effectively leverage ML algorithms (Zhang., 2021). The interpretability and transparency of machine learning (ML) algorithms present a significant challenge, particularly when it comes to their application in fields like plant breeding. While these algorithms offer powerful predictive capabilities, their inherent complexity can hinder effective communication between ML experts and domain-specific practitioners such as plant breeders (Libbrecht., 2015; Zhang., 2021). Consequently, it is widely recognized that no single ML algorithm is universally suitable for all situations (Yoosefzadeh-Najafabadi et al., 2023).

I.9.2.2 Machine Learning: Basis and Function

Supervised learning constitutes a fundamental pillar of machine learning, where algorithms are trained using labeled data to make predictions (Hesami and Jones., 2020). Its capabilities have enabled significant advancements in predicting complex traits, identifying relevant genomic regions, and optimizing genotype selection (Yoosefzadeh-Najafabadi et al., 2022).

The Supervised Machine Learning algorithm can be broadly classified into Regression and Classification Algorithms (Figure I.9). Classification algorithms are used to predict the output for the categorical values, while in regression algorithms are used to predicted the output for continuous values (Yoosefzadeh Najafabadi et al., 2023). All algorithms learn from the training dataset and apply them to the test dataset for prediction, therefore a computer program is trained on the training dataset and based on that training, it categorizes the data into different classes (Figure I.9).

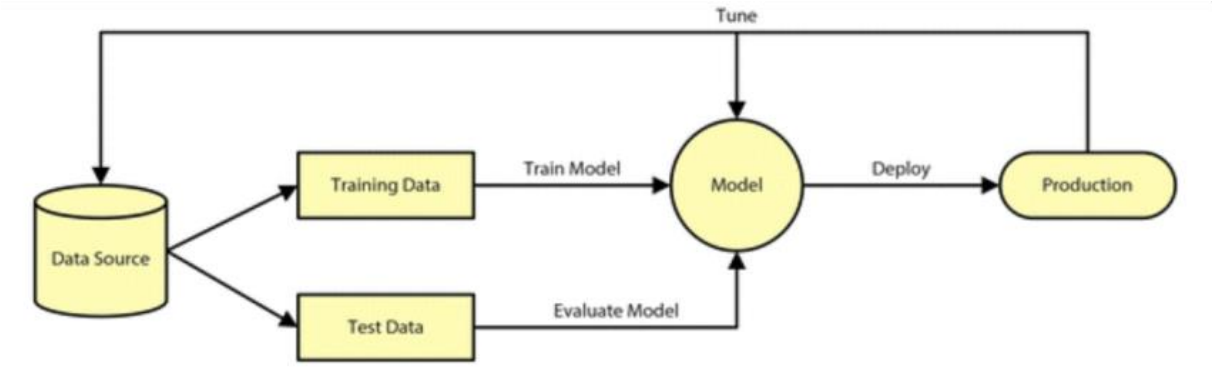


Figure I.9. The workflow of supervised machine learning algorithms (Batta Mahesh., 2020).

I.9.2.3 Analyzing Machine Learning Models Performance

Assessing the performance of machine learning (ML) algorithms on a given dataset is a critical step in the process of building and deploying effective predictive models. This process involves various techniques and metrics to quantify how well an algorithm is performing and to compare different algorithms' performance objectively (Libbrecht and Noble., 2015). The evaluation process commences by selecting how to divide datasets into training, validation, and testing sets through the utilization of the subsequent techniques:

- **Train/Test Splitting:** This method entails splitting the dataset into two partitions - one for training and one for testing. The model is trained on the training portion and subsequently assessed on the testing portion.
- **Cross-Validation:** Involving the division of the dataset into k subsets, cross-validation comprises training and evaluating the model k times. During each iteration, one of the k subsets functions as the test set, while the remaining k-1 subsets are employed as training sets (Browne., 2000).
- **Bootstrap Method:** This approach involves generating random samples with replacement from the original dataset. The model is then trained and evaluated using these generated samples (Johnson., 2001).
- **Leave-One-Out Cross-Validation (LOOCV):** Here, the dataset is partitioned into n folds. The model is trained and evaluated n times, with one data point serving as the test set in each iteration, while the other n-1 data points are used for training (Cawley and Talbot., 2003).

When it comes to gauging the effectiveness of machine learning techniques, there exist numerous methods for assessing errors. These approaches aid in comprehensively evaluating

the predictive accuracy of machine learning algorithms. In classification models, an array of techniques is employed to assess the model's efficacy in making predictions across various classes. These methods, including accuracy, precision, recall, and the F1 score, provide insightful metrics for evaluating the performance of classification algorithms (Huang and Ling., 2005).

The accuracy of a machine learning algorithm is quantified through its accuracy, which signifies its proficiency in predicting classes within a provided dataset (Huang and Ling., 2005). This is commonly represented as a percentage and is calculated by dividing the number of correct predictions made by the total number of predictions.

Utilizing the concept of **precision** provides a focused perspective on the model's ability to accurately identify instances of a specific class within a dataset (Huang and Ling., 2005). This metric measures the proportion of true positive predictions divided by the total number of positive predictions. The mathematical calculation of precision is defined through an equation (Galdi and Tagliaferri., 2018).

$$\textbf{Precision} = \frac{\textbf{TP} + \textbf{TN}}{\textbf{TP} + \textbf{FP} + \textbf{TN} + \textbf{FN}}$$

Where, TP stands for true positive samples, TN represents true negative samples, FP is false positive samples, and FN stands for false negative samples in the confusion matrix.

Recall, also known as **sensitivity** or true positive rate, is a crucial metric that assesses a model's effectiveness in retrieving all relevant instances from a dataset. It revolves around the notion of identifying true positives in relation to the actual positives present within the data. In essence, recall gauges the model's capacity to capture and recall instances that truly belong to a specific class. The calculation of recall involves dividing the number of relevant instances that the model correctly identifies by the total count of relevant instances present in the dataset. This can be formulated using equation as followed:

$$\textbf{Recall} = \frac{TP}{TP + FN}$$

This equation quantifies the ratio of true positives to the sum of true positives and false negatives. Higher recall values indicate that the model has the ability to find a larger proportion of the relevant instances in the dataset. In other words, the model is effectively minimizing the number of false negatives, where actual positives are incorrectly identified as negatives (Galdi and Tagliaferri., 2018)

I.9.2.4 Various supervised machine learning algorithms in plant breeding

There are several machine algorithms that have been used in plant breeding, including:

- **Support vector machine (SVM)**

A support vector machine (SVM), is a machine learning algorithm which can be applied to classification and regression problems and that learns by example to assign labels to objects (Boser., 1992; Pisner et al., 2020). SVMs have been successfully used in a wide variety of biological applications (Golub et al., 1999; Furey et al., 2000; Noble., 2004). Identification a hyperplane in an N-dimensional space (N is the number of features) that categorizes the data points clearly is the purpose of the support vector machine algorithm. The fundamental concepts behind the SVM algorithm may be described without ever reading an equation. For SVM classification, one only needs to understand four fundamental ideas: the separating hyperplane, the maximum-margin hyperplane, the soft margin, and the kernel function (Hearst., 1998; Noble., 2006).

- **Random Forest algorithm**

Random forests also known as random decision forests developed by Breiman in (2006) and Cutler in (2012) mixes the output of various decision trees to produce a single outcome. Its widespread use is motivated by its adaptability and usability because it can solve classification and regression issues. There are three key hyperparameters for random forest algorithms that must be set prior to training, including node size, the number of trees, and the number of features sampled. The random forest algorithm is composed of a collection of decision trees, and each tree in the ensemble contained a data sample drawn from a training set with replacement, named the bootstrap sample (Kam., 2002). One-third of that training sample, or the "out-of-bag" (oob) sample, is set aside as test data. The dataset is subsequently given a second randomization injection by feature bagging, increasing dataset diversity and decreasing decision tree correlation. After that, the prediction is finally validated using the oob sample. The prediction will be determined differently depending on the type of issue (Breiman., 2006). Figure (I. 10) illustrates the scheme of random forest algorithm. The final prediction is obtained by taking a majority vote of the predictions from all the trees in the forest.

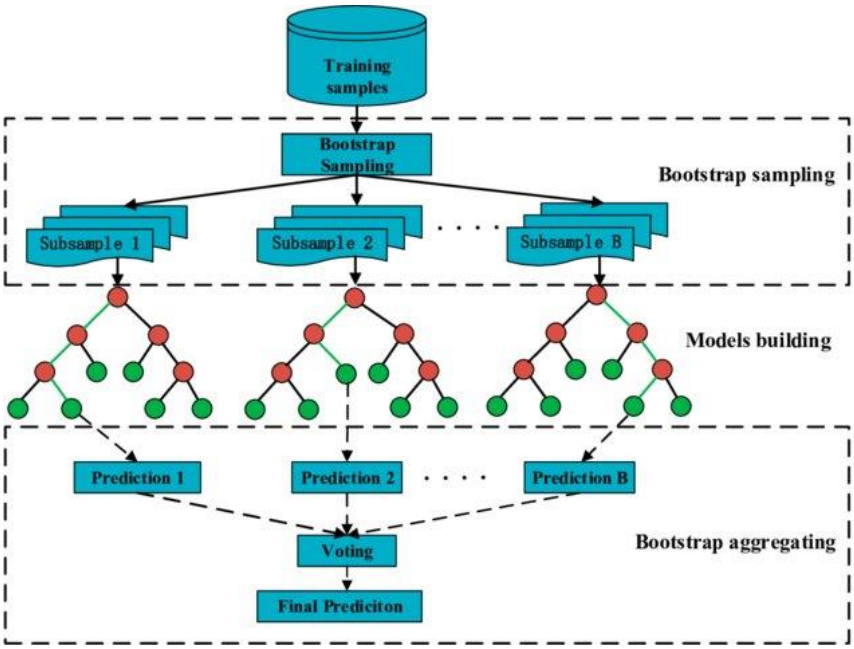


Figure I.10. The scheme of random forest algorithm (Wang et al., 2019)

- **K-nearest neighbors (KNN) algorithm**

K-Nearest Neighbors is one of the simplest and non-parametric algorithm developed by Fix and Hodges in (1951). The principe of KNN is to save the training dataset rather than learning from it immediately**.** The KNN algorithm assumes that similar things are located nearby. Further, KNN algorithm can be applied to solve both classification and regression problems. The basic kNN has usually only a single parameter (k) which is relatively easy to tune (Kotsiantis., 2007). The k parameter in the k-NN technique specifies how many neighbors will be considered to determine the classification of a specific query point, the k value is also a user-defined constant (Kotsiantis., 2007). The optimal value of k depends on the data, typically higher values of k reduce the impact of noise on classification (Everitt., 2011).

Chapter II: Assessment and modeling using machine learning of resistance to scald (*Rhynchosporium commune*) in two specific barley genetic resources subsets

II.1. Introduction

Barley (*Hordeum vulgare* L.) remains one of the most important cereal crops since its domestication in the "Fertile Crescent" 10,000 years ago (Harwood., 2019). It ranks fourth among cereals in terms of production and acreage and is grown mainly in Russia, Australia, Europe, United Kingdom, Canada, North America, and Asia (FAOSTAT., 2018). Barley is a major crop in North Africa and West Asia, and it is grown on an average ~ 3 million ha in the dry areas of Morocco and Ethiopia (FAOSTAT., 2016).

Barley is a multiple purpose crop used mainly as feed (green forage, straw, and grain), food, malt, and brewing. Due to its health benefits, it has become an important ingredient of the modern human diet in many developing as well as developed countries (Ullrich., 2010). Particularly in Japan, more than 90% of barley is used as human food (Aoki., 2011). In Morocco, Ethiopia, India and China, barley grain is still widely consumed (Ashman and Beckley., 2006). Recently, barley grain is being used in renewable energy for the production of biofuels (Griffey et al., 2010). Barley has a great potential to adapt to a wide range of environmental conditions, ranging from deserts, to high latitudes and altitudes (Daiand Zhang., 2016). Around the globe, barley can grow in the spring and winter seasons (Cuesta-Marcoset al., 2016). However, barley productivity and quality are affected by several biotic factors including viruses, fungi, and bacteria (Pessarakli., 2020).

Among these phytopathogens, *Rhynchosporium commune* (formerly *R. secalis*), is one of the most serious and destructive fungi causing a foliar disease known as scald or leaf blotch. This disease is found in all continents but is more frequent and devastating under cool and semi-humid barley growing regions (Zhan., 2008). Yield losses due to scald can vary from 10 to 70% (Zhan., 2008; Paulitz and Steffenson., 2010; Jabbari., 2012; Karakaya et al., 2014). Furthermore, Home-Grown Cereals Authority in the United Kingdom reported that despite the use of chemical fungicides, *R. commune* causes annual yield losses worth £4.8 million (Ullrich., 2010).

Owing to its high genetic diversity and spontaneous mutations, *R. commune* populations can evolve rapidly (Abang, et al., 2006), and has the potential to quickly adapt and overcome deployed host plant resistance (Williams., 2003; Zaffarano., 2006), making its control more challenging. Linde et al in (2003) found 76% of the global genetic diversity of the pathogen

within a single barley field. *R. commune* is an imperfect fungus, and its perfect stage has not been reported. Pathogens with mixed reproduction systems pose serious threats to longevity of resistance genes. A sexual reproduction can be predicted based on equal frequency of two matting types in the population. In Turkey, Çelik Oğuz et al in (2021) reported 35% of the isolates with MAT1-1 and 65% of the isolates with MAT1-2 which hints asexual reproduction. In addition, other matting type studies indicate asexual reproduction in Iran and Syria, while in Switzerland, Australia, Ethiopia, South Africa, Scandinavia and California, sexual reproduction has been predicted (Linde., 2003; Seifollahi., 2018). For developing control strategy, genetic diversity and its distribution among populations may be helpful. Furthermore, *R. commune* populations can adapt to abiotic selection pressure, comprising the variation of temperature and global warming (Stefansson., 2013). Another challenge complicating the control of scald disease is its ability to survive, colonize and sporulate asymptomatically on the resistant varieties, which can be a source of inoculum for the next cropping season (Atkins et al., 2010). Many studies showed that polymerase chain reaction (PCR) can detect symptomless presence of *Rhynchosporium* in the seeds (Lee., 2002), which allows the transmission of the pathogen (Fountaine., 2005).

Virulence changes in pathogen populations affect the disease resistance status of the host plant genotypes. It is necessary to understand the evolutionary forces which impact pathogen biology in terms of emergence of individual genotypes with altered virulence. Conversely, plant genetic resources should be screened with newly emerged virulent pathotypes for effective management of the disease. Several factors such as large population size, frequency-dependent selection, spontaneous mutation, gene flow, sexual reproduction and asexual recombination could explain the high genetic diversity of *R. commue* populations (Avrova and Knogge., 2012; Oğuz., 2021). Different studies have reported 272 pathotypes based on responses of diverse isolates on selected barley genotypes (Fowlerand Owen., 1971; Ali., 1976; Ceoloni., 1980; Abbott et al., 1991; Tekauz., 1991; Goodwin, 2002; Azamparsa and Karakaya., 2020; Oğuzand Karakaya., 2021). Azamparsa and Karakaya in (2020) grouped 52 scald isolates into 30 pathotypes based on the screening on 17 differential barley genotypes. None of the pathotypes was virulent on all differentials and two cultivars, Jet and Abyssinia, were the most resistant with a mean disease score of 0.2–0.3 on a scale of 0–4. Therefore, a continuous search for new effective resistance genes and their deployment through breeding is needed to cope with the emerging new virulent pathotypes of leaf scald pathogen.

The use of fungicides and cultural practices are not efficient in sustainable management of leaf scald disease, and resistance to multiple fungicides has been reported by many researchers

(Brunner., 2016; Mohd-Assaad., 2016). *R. commune* has developed insensitivity to demethylation inhibitor fungicides e.g., methyl benzimidazole carbamates (Avrova and Knogge., 2012), and to azole-based fungicides through mutation in multiple loci (Mohd-Assaad., 2016). Therefore, genetic resistance remains the most economical and environmentally friendly way to control scald disease (Oxley., 2003). Thus, genetic resources, such as accessions of barley landraces and wild barley species held in the gene banks are key to crop improvement efforts, as they allow continuous supply of traits sought by the breeders, including genes for resistance/tolerance to major abiotic and biotic stresses (Qualset., 1975). Wild barley (*Hordeum spontaneum*) offers great potential as source of resistance compared to landraces. The response of 198 landraces and 104 *H. spontaneum* accessions from Turkey revealed that only 0.5% (1 accession) of the landraces were resistant to six isolates of scald, but 26% (27 accessions) of *H. spontaneum* accessions were resistant to all isolates tested (Azamparsa., 2019). Similarly, Rehman et al (2021) reported that 58% (66) of *H. spontaneum* accessions from ICARDA genebank were resistant to scald under field conditions in Morocco. Silvar et al in (2010) screened 159 lines issued from a core collection of Spanish barley landraces and found 26% being resistant to scald. Yitbarek et al in (1998) found sources of resistance to scald in the Ethiopian germplasm with a higher rate of resistance in accessions originated from higher altitudes. Furthermore, van Leur et al in (1989) found 13% lines being resistant to scald from a set of 100 lines derived from landraces collected from different regions of Syria and Jordan. They observed more variation within landraces and among collection sites, with more prevalence of resistant accessions from cooler environments. Furthermore, Düşünceli et al (2008) reported 8 out of 683 barley genotypes resistant at the seedling as well as at the adult plant stage.

ICARDA has the global mandate for the improvement of barley (ICARDA., 2018), and its genebank has one of the largest collections of barley worldwide totaling around 32,800 accessions, including more than 2500 accessions of wild *Hordeum* species (Genesys PGR., 2018). ICARDA distributes to collaborators the elite germplasm in the form of international nurseries, and accessions of genebank based on requests and using the Standard Material Transfer Agreement (SMTA). The distribution of accessions was done in the past either using random sampling or using core collections with the latter attempting to include 90% of the diversity in 5–10% of the total holdings (Hintum., 1997). The Generation Challenge Program (GCP) proposed the development of a reference set representing most of the genetic diversity in 10% of the core collection using molecular markers. ICARDA with the Australian and the Russian collaborators developed an approach named “Focused Identification of

Germplasm Strategy (FIGS)", which is either based on filtering or predictive modeling, to construct best-bet subsets for targeted traits (Mackay and Street., 2004). The predictive modeling approach allows to develop algorithms linking the sought plant traits to geographic, agro-climatic, and edaphic variables, which are used to construct manageable subsets for efficient evaluation and mining of genetic resources (Azough., 2019). The relationship between the trait and the environment was shown in case of barley by Endersen in (2010) and was tested for salinity tolerance (Hijmans., 2003), and for net blotch resistance (Endresen., 2011). Furthermore, FIGS was successful in identifying sources of resistance to the Russian wheat aphid and Sunn pest in wheat which could not be identified using random sampling of larger subsets (El Bouhssini., 2009; El Bouhssini., 2011). In addition, new alleles for resistance to powdery mildew in wheat (Bhullar., 2009) were identified from a targeted FIGS subset.

The present study aims at the identification of sources of resistance to scald in GCP and FIGS subsets, and to assess different predictive models for efficient mining of genetic resources for resistance to scald.

II.2. Material and Methods

II.2.1. Germplasm used

A total of 292 genotypes from the ICARDA genebank were evaluated for scald resistance including 101 accessions selected using FIGS approach (FIGS subset), and 191 accessions from the Generation Challenge Program reference set (GCP).

The filtering approach was used to develop FIGS subset based on the following parameters:

- Count number of days where the average daily temperature is between 8 and 12 °C, 10 days before the onset of the growing period and up to 15% into the vegetative phase.
- Remove sites with zero count from step 1.
- Sum daily rain for 10 days before the onset of the growing period up to 10% into the vegetative phase.
- Normalize both variables (steps 1 and 2) to range 0–1 for each site.
- Add variables to create index 1.
- Rank based on index 1 and remove the bottom 25 percent of sites.

For the remaining sites, the following was done:

- From 10% into the vegetative phase until onset of grain filling divide into 3 separate sub-phases of equal length.
- For each sub-phase count the number of days where the average daily temperature is between 15 and 20 °C.

- For each sub-phase determine the amount of precipitation.
- Remove sites if any of the variables = 0 (3 count variables and 3 precipitation variables).
- Normalize each variable for a range between 0 and 1.
- Add each variable and then add index 1 to create index 2.
- Rank sites using index 2 from largest to smallest.

Depending on the required subset size and the number of remaining candidate sites, proceed with the selection of accessions.

- If there are fewer candidate sites than the required set size, choose one accession in a cyclic manner working down the rank and then repeat starting at the top of the rank with sites that still have accessions after each pass. Alternatively, starting from the top ranked site, multiple accessions per site could be chosen until desired set size is achieved.
- If there are more sites than the desired set size, then one accession could be chosen randomly from each site starting at the top ranked site until the desired set size is reached. Alternatively, this approach could be taken after one candidate accession is donated by each country represented in the candidate site list.

II.2.2. Fungal isolates and inoculation

Scald infected barley leaves were collected during the disease surveys conducted from 2015 to 2018 in naturally infected barley fields from different agro-ecological zones of Morocco including Marchouch and Sidi Allal Tazi research stations. Infected barley leaves were dried in paper envelops at room temperature for 4–5 days before their storage at 4 °C till further use. Air dried leaves were cut into 2 mm segments and soaked in sterile distilled water for 15 min followed by surface sterilization using 50% ethanol for 15 s and 10% sodium hypochlorite solution for 30 s, rinsed with sterile distilled water three times, and dried up within two layers of sterile Whatman filter paper. Then the leaf segments were incubated on Lima Bean agar (LMA) supplemented with Kanamycin and Streptomycin (50 mg per liter) at 14 °C in darkness for two weeks until the sporulating white or pink colonies were visible. A total of 50 single conidial isolates were prepared and stored at 80 °C freezer for long term storage. Based on race analysis, 12 pathotypes were revealed and two scald isolates with a wide virulence spectrum (Unpublished data), Marchouch (MCH-SC) and Sidi Allal Tazi (SAT-SC), were used for seedling screening in the greenhouse. While for adult plant screening, inoculum composed of a mixture of 15 pathotypes from different agro-ecological zones of Morocco was used.

II.2.3. Seedling screening (SRT)

About 4–5 seeds of each accession were sown in peat moss supplemented with 14-14-14 NPK in a single cone of 3.8 cm diameter and 14 cm depth (Stuewe & Sons, Inc., Oregon, USA) in two replications. The seedlings were grown in a growth chamber at a photoperiod of 16 h light/8 h dark at 20 ± 1 °C. At the two leaves stage, inoculation was performed separately with two virulent isolates of barley leaf scald (SC-511, and SC-1122). The inoculum was prepared from 12 to 14 days old LBA plates by rubbing the agar surface gently followed by filtration with two layers of cheese cloth. Spore suspension of 5×10^5 conidia/ml supplemented with surfactant Tween 20 (0.01%) was sprayed with a hand-held sprayer till run off. The inoculated seedlings were kept at 100% relative humidity in the dark in the growth chamber for 72 h at 15 °C to enhance spore germination. Afterward, the seedlings were transferred to the greenhouse with a light/dark regime of 16/8 h at 16 ± 1 °C, respectively.

The disease evaluation at the seedling stage was carried out after 16 days post-inoculation. Assessment of plant infection type and severity was done based on scoring scale (0–5) of Salamati and Tronsmo in (1982) considering lesion morphology, size, chlorosis and necrosis. Where 0 = Immune (I), 1 = resistant (R), 2 = moderately resistant (MR), 3 = moderately susceptible (MS), 4 = susceptible (S), and 5 = highly susceptible (HS). For further analysis, the genotypes were categorized into either resistant (IR of 0, 1, 2) or susceptible (IR > 3, 4, 5) group.

II.2.4. Adult plant screening (APS)

The adult plant screening was conducted in Ethiopia and Morocco. In Morocco, the trials were conducted at the INRA experimental stations of Marchouch (MCH; 33° 56 N, 6° 63 W) and at Guich (33° 58 N, 6° 51 W) during the cropping season of 2018. Each entry was seeded in 1 m length row with a row spacing of 0.5 m in an augmented design. Two susceptible (Tocada and Tissa) and resistant (ICARDA4 and Atlas 46) checks were planted after every 10 accessions and each block was surrounded by a perpendicular border row, composed of a mixture of scald susceptible genotypes (Tiddas, Aglou, Tissa, Adrar, Shepherd, Fleet, Baudin and Alester), to maintain high disease pressure. Artificial field inoculations were performed four times starting from Zadoks scale (Zadoks., 1974) GS30 at an interval of 10–12 days apart in the evenings with inoculum composed of spore suspension composed of a mixture of 50 *R. commune* isolates collected from different agro-ecological zones of Morocco using a knapsack sprayer. In addition, scald infected barley residues were spread uniformly in the field. Following inoculations, the covering of spreader rows with a plastic sheet overnight and the frequent use of a mist system with overhead sprinklers ensured good disease establishment and uniform

spread of the disease. In Ethiopia, the experiment was conducted in Holetta experimental station (9° 00′ N and 38° 30′ E) during 2017 and 2018 cropping seasons using the same design as described above. There, the environmental conditions were favorable to allow a high natural infection level of scald for efficient screening of the germplasm. The disease severity was assessed at GS 73–75 using 0–9 scale (Saari and Prescott, 1975). Genotypes were classified in the following categories: Resistant (0–2), moderately resistant (3–4), moderately susceptible (5–6), susceptible (7–8), and highly susceptible (9).

II.2.5. Data analysis

The statistical analysis was performed using R software (Team, R., 2018). The disease assessment data from the seedling and adult plant stage were tested for statistical association between the sub-setting approaches using χ^2 tests of independence with significance level ($\alpha = 0.05$). The equation used to calculate chi-square is as follows:

$$\chi^2 = \sum_{i=1}^{r} \sum_{j=1}^{c} \frac{(O_{ij} - E_{ij})}{E_{ij}} \quad (1)$$

The equation for calculating expected values in a test of independence is as follows:

$$E_{ij} = \frac{\sum_{k=1}^{c} O_{ij} \sum_{k=1}^{r} O_{kj}}{N} \quad (2)$$

where is the E_{ij}= expected value, $\sum_{k=1}^{c} O_{ij}$ is the Sum of the i th column, $\sum_{k=1}^{r} O_{kj}$ is the Sum of the kth row, N is the total number.

The expected values for the test of goodness were calculated using following equation:

$$E_i = np_i \quad (3)$$

Where E_i is the expected value, n is the total sample size, and p_i is the hypothesized proportion of observations in level *i*.

To find out the differences between FIGS and GCP subsets in terms of reaction to scald, the test of goodness of fit using χ^2 test at a significance level ($\alpha = 0.05$) was used where GCP was simulated to a random sample. Both tests were done using different groupings of reactions, all six classes (I, R, MR, MS, S and HS), three classes (I + R, MR + MS, S + HS) and two classes (I + R + MR and MS + S + HS).

II.2.6. Modeling of the reaction to scald disease

The machine learning approach using the obtained SRT and APS disease screening data was used to find out the best models that link adaptive traits, environments (and associated selection pressures) with genebank accessions. We used environmental data from WorldClim1 databases as predictors. WordClim is an open access database providing global climatic layers describing past climatic profiles of collection sites and intended for spatial modeling or mapping. It includes average, monthly minimum and maximum temperatures, precipitation, and bioclimatic variables (Ick and Hijmans., 2017).

The following machine learning algorithms were used: K-nearest neighbors (KNN) (Kotsiantis., 2007), Support Vector Machines (SVM) (Hsu., 2003), Random Forest (RF) (Breiman., 2001), and Bagged Carts (BCART) (Kołcz., 2000). Each machine learning model was tuned to select best tuning parameters using a training set (70% of the total set) and then the best model was selected between different machine learning models based on several metrics including accuracy, specificity, and Kappa. The modeling metrics were computed on the test set (30% of the total set). In this study, R language and caret library were used for machine learning. Models were tuned for parameter's optimization and trained on 70% of the data and tested with 10 cross validation folds and 100 replications. Modeling was done for the two isolates for the seedling stage using average and high scores separately. For the adult plant reaction, modeling was done for the combined multi-locations data sets and for each location separately.

II.3. Results

II.3.1. Reaction of GCP and FIGS subsets to scald disease

Scald infections were successful under both controlled and field conditions as shown by the uniformity of the infection of the susceptible accessions as well as the checks. The averages scores of susceptible (Tocada and Tissa) and resistant (ICARDA4 and Atlas 46) checks at the adult plant stage were 8 and 1, respectively. While at the seedling stage, average scores of resistant and susceptible checks for isolate SC-511 were 0 and 5, and for the isolate SC-1122 were 1 and 4, respectively.

The two subsets (FIGS and GCP) included sources of resistance to scald at both the seedling and adult plant stages, but their percentages differed based on isolates at the seedling stage and based on location and pathogen populations under field conditions. At the seedling stage, GCP subset showed 6% and 16% resistant accessions, and 79% and 54% susceptible accessions to scald isolates SC-511 and SC-1122, respectively. For FIGS subset, 12% and 34% of the

accessions were resistant, while 60% and 21% were susceptible to SC-511 and SC-1122 isolates, respectively (Fig. II.1.A, B). Different percentages for the moderately resistant and moderately susceptible classes were found among the two subsets for both scald isolates tested. FIGS subset had 8% more accessions with MR and MS reactions compared with GCP for the SC-1122 isolate. For isolate SC-511, 4% of GCP and 2% of FIGS accessions were MR while 12% and 26% showed MS reaction, respectively. Isolate SC-511 showed less percentage of accessions with immune, resistant, and moderately resistant reaction and a higher percentage of susceptible accessions compared to isolate SC-1122 (Fig. II.1).

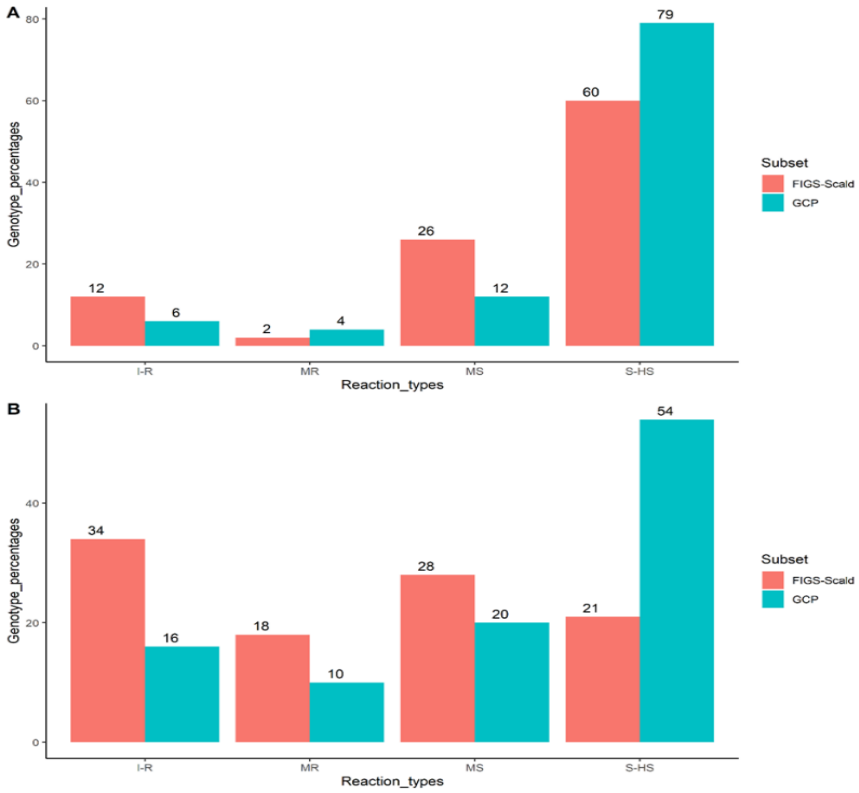


Figure II.1. Seedling reaction to scald isolates 511 (A) and 1122 (B) of barley accessions of FIGS and GCP subsets under control conditions (in percent).
I–R Immune and resistant, MR moderately resistant, MS moderately susceptible, S-HS susceptible and highly susceptible.

At the adult plant stage, the percentage of accessions for scald disease severity differed based on the location, year, and subsets. The highest percentages of resistant accessions for both subsets (65% for GCP, and 82% for FIGS) were observed at Guich-Morocco 2018, and the lowest percentages at Holetta 2017 (25% for GCP, and 22% for FIGS) (Figure II.2). For Marchouch 2018, 37% of GCP and 51% of FIGS were resistant, while for Holetta-nursery 2018, these respective percentages were 46% and 53%, and 55% and 61% for Holetta-quarantine

2018. The highest percentages of susceptible (32% for GCP, and 44% FIGS), and highly susceptible accessions (10% for GCP, and 5% for FIGS) were identified at Holetta-nursery 2017 for both subsets, while for all the other environments these classes of reaction ranged from 0 to 10%.

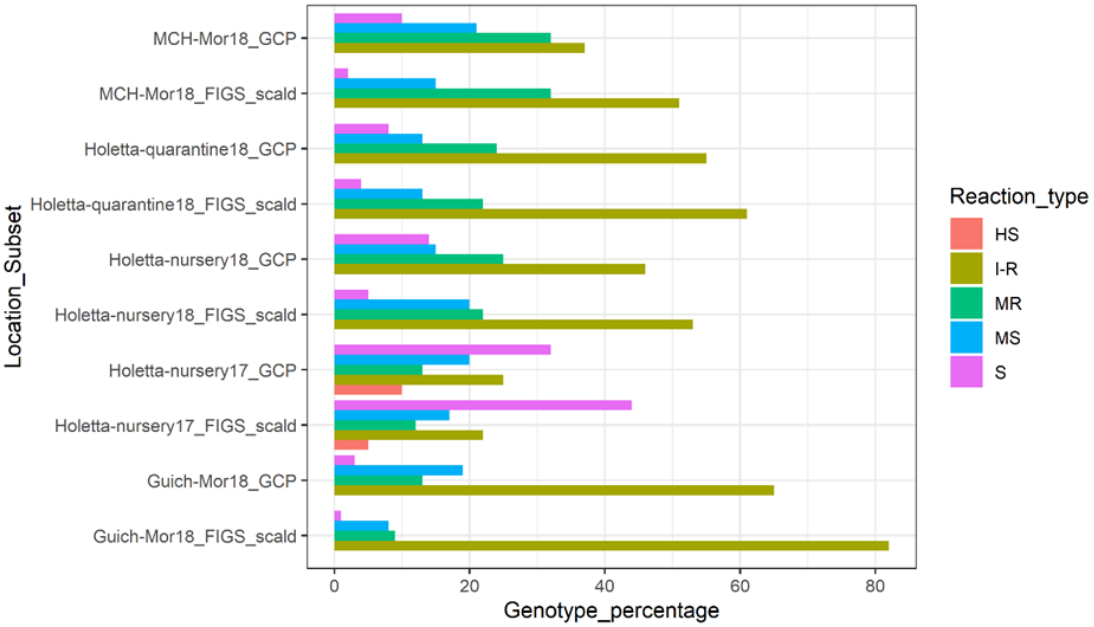


Figure II.2. Adult plant reaction of GCP and FIGS barley subsets (in percent) to scald populations under field conditions. I–R Immune and resistant, MR moderately resistant, MS moderately susceptible, S susceptible, HS highly susceptible.

When the disease scoring is grouped into three classes, (I + R, MR + MS, S + HS), the highest percentage of resistant accessions were found in the case of Guich Morocco-2018, and the lowest at Holetta-nursery 2017. For the moderately resistant and moderately susceptible classes, the GCP and FIGS subsets had similar percentages except at Guich-2018 where GCP (33%) had almost twice as much as FIGS (17%) (Figure II.3).

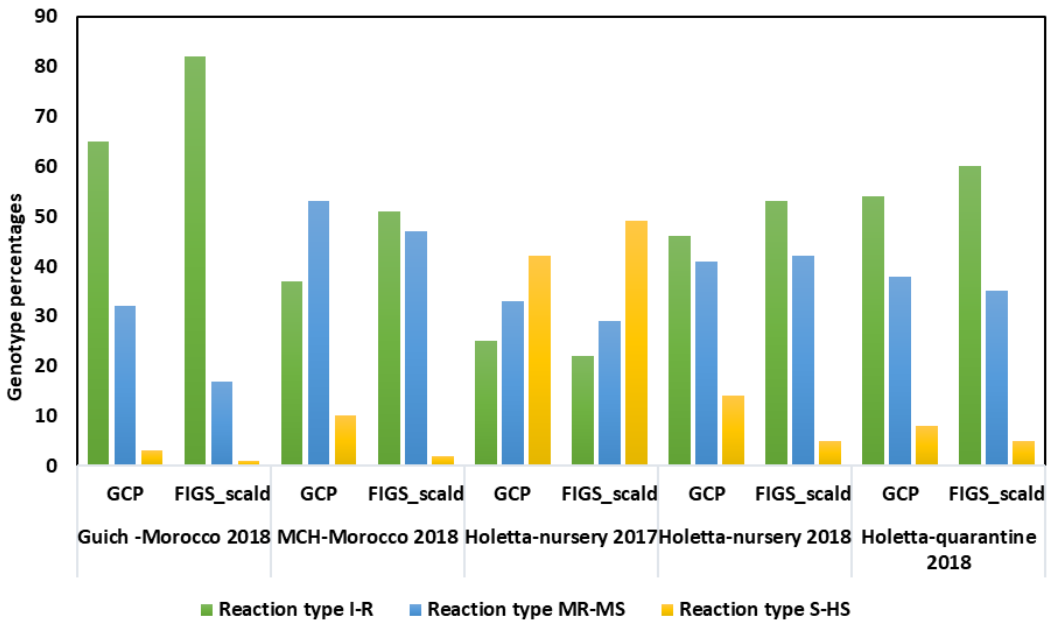


Figure II.3. Adult plant reaction of GCP and FIGS barley subsets (in percent) to scald populations under field conditions when grouped into 3 classes (I-R: Resistant class; MR-MS: Moderate class; S-HS: Susceptible class).

II.3.2. Comparison of GCP and FIGS subsets

The tests of independence showed that the reaction to scald is dependent on the subsets when tested in the field at Guich and Marchouch in Morocco with $\chi 2$ P-values < 0.01 (Table II.1). However, this dependency was not observed when the subsets were tested against the scald natural populations in Ethiopia. The same conclusions were confirmed when different classes of disease reactions were combined (Annex II.1). In case of dependency, FIGS appeared to deviate from theoretical distribution and had higher percentages of accessions with resistant and moderately resistant reactions and lower percentages of susceptible and highly susceptible accessions. At the seedling stage, the reaction types to both isolates were dependent on the subsets for all classes of reactions (P values < 0.01), except for the isolate SC-511 for the grouping (I + R + MR) and (MS + S + HS) with a p- value of 0.201 (Table II.1).

Table II.1: Test of independence of adult plant reaction of FIGS and GCP barley subsets to scald evaluated under field conditions in Ethiopia (2017–2018), and in Morocco (2018) for each disease reaction class.

Locations	Sets	Reaction type						χ^2 (P-value)
		I	R	MR	MS	S	HS	
Guich-Morocco 2018	GCP	58	66	26	36	5	0	12.664 (P = 0.013)
	FIGS_ scald	48	35	9	8	1	0	
MCH-Morocco 2018	GCP	6	64	62	40	19	0	12.099 (P = 0.017)
	FIGS_ scald	8	44	32	15	2	0	
Holetta-nursery 2017	GCP	24	24	24	39	61	19	5.756 (P = 0.331)
	FIGS_ scald	9	13	12	17	44	5	
Holetta-nursery 2018	GCP	38	48	47	30	26	0	6.635 (P = 0.156)
	FIGS_ scald	26	27	22	20	5	0	
Holetta-quarantine 2018	GCP	42	61	46	25	15	0	5.231 (P = 0.264)
	FIGS_ scald	33	27	22	13	4	0	

I Immune, R resistant, MR moderately resistant, MS moderately susceptible, S susceptible, HS highly susceptible.

For the test of goodness of fit, highly significant differences between FIGS and GCP subsets were declared based on a comparison of different classes of disease reactions at Guich and Marchouch (2018) in Morocco with P values < 0.01. These differences were significant (P-value = 0.033) in case of the trial in Holetta-nursery 2018, but not in Holetta-nursery 2017 (Annex II.2). When the differences were significant, FIGS subset showed higher percentages of resistant classes and lower percentages of susceptible classes than GCP subset at the adult plant stage. When the tests were done at the seedling stage using both isolates, the differences were highly significant between FIGS and GCP subsets for all groups of disease reaction classes except for the isolate SC-511 for the grouping (I + R + MR) and (MS + S + HS) with a p-value of 0.091 (Table II.2).

Table II.2: $\chi 2$ tests of independence and of goodness of fit of different groups of disease reaction classes to two scald isolates (511, 1122) under controlled conditions using GCP and FIGS subsets of barley.

Isolates	Set	Group of 6 classes	Group of 2 classes	Group of 3 classes
		χ^2 (P-value)	χ^2 (P-value)	χ^2 (P-value)
Test of independence				
SC-511	GCP	21.352 (< 0.01)	1634 (P = 0.201)	12.107 (< 0.01)
	FIGS_scald			
SC-1122	GCP	35.140 (< 0.01)	18,265 (< 0.01)	30.290 (< 0.01)
	FIGS_scald			
Test of goodness of fit				
SC-511	GCP	39.944 (< 0.01)	2.851 (P = 0.091)	22.143 (< 0.01)
	FIGS_scald			
SC-1122	GCP	60.501 (< 0.01)	18.265 (< 0.001)	46.698 (< 0.01)

Group of 6-classes includes reaction types I, R, MR, MS, S, HS, group of 2-classes include I + R + MR and MS + S + HS, group of three classes include I + R, MR + MS, and S + HS.
FIGS Focused Identification of Germplasm Strategy subset, GCP Generation Challenge Program reference set.

II.3.3. Prediction of reaction to scald using machine learning approach

In our present study the performance of predictive models varied depending on the environment at the adult plant stage, and on the isolates at the seedling stage. At the seedling stage, the two modeled classes were not balanced for both high and averages scores (Table II.3). The isolate SC-1122 showed a better balance than isolate SC-511 between resistant and susceptible classes. High score exhibited a higher but non-significant accuracy than the average score for both isolates. However, models for average accuracy were significantly higher than Null accuracy with 0.89 and 0.64 for isolate SC-511 and SC-1122, respectively. Models for both isolates, and both scores were not able to identify efficiently resistant accessions due to low sensitivity with maximum value of 0.25. At the adult plant stage for the combined field data, only two models showed significant and accurate results, BCART and RF with an accuracy of 0.73 and 0.72, respectively (Annex II.3).

Table II.3: Modeling performance from the best machine learning algorithm for seedling data for high (HS) and average (AS) disease scores for scald disease in barley set.

Best model	**SC-511 HS**	**SC-511 AS**	**SC-1122 HS**	**SC-1122 AS**
	BCART	**BCART**	**KNN**	**SVM**
Sensitivity	0.20	0.11	0.25	0.25
Specificity	0.96	1.00	0.84	0.88
Precision	0.25	1.00	0.36	0.54
Accuracy	0.91	0.89	0.68	0.64
Kappa	0.17	0.18	0.10	0.14
Accuracy lower	0.82	0.80	0.57	0.53
Accuracy upper	0.96	0.95	0.79	0.75
Accuracy null	0.93	0.88	0.74	0.63
Mcnemar (P-value)	1.00	0.01	0.31	0.01

BCART Bagged Carts, KNN K-nearest neighbors, SVM support vector machine.

Table II.4: Performance metrics for the best machine learning models for scald reaction of barley at adult plant stage in different environments.

Best model	**GUICH**	**MCH**	**Holetta17**	**Holetta18**
	BCART	**RF**	**SVM**	**KNN**
Sensitivity	0.94	0.95	0.41	0.73
Specificity	0.23	0.53	0.77	0.25
Precision	0.86	0.81	0.52	0.63
Accuracy	0.82	0.81	0.63	0.55
Kappa	0.21	0.53	0.19	− 0.02
Accuracy lower	0.72	0.75	0.51	0.43
Accuracy upper	0.90	0.87	0.74	0.67
Accuracy null	0.83	0.69	0.62	0.63
Accuracy (P-value)	0.69	0.00	0.46	0.94

BCART Bagged Carts, RF Random Forest, SVM support vector machine, KNN K-nearest neighbors, MCH Marchouch station.

Specificity was very low-to-medium for all the four assessed models, and the Null accuracy was higher than 0.5 and reached to 0.67. All models efficiently identified resistant accessions

with specificities higher than 0.88. Modeling of field data per location/environment (Table 4) showed different patterns. Accuracy from modeling Marchouch was significantly higher than Null model with an accuracy of 0.81 and Kappa of 0.53.

II.4. Discussion

The good development of scald under natural infection in Holetta-Ethiopia and under artificial inoculation with the use of overhead sprinklers irrigation system in Morocco allowed reliable screening of FIGS and GCP subsets. Over the two cropping seasons, scald appeared as a permanent threat to barley in Ethiopia while in Morocco its natural development was more sporadic in time and space. The surveys conducted in North African countries during 1990–1994 through UNDP-ICARDA project on cereals and food legume diseases showed that scald is a major disease of barley in Tunisia, but in Morocco, infections were observed only once out of five years (unpublished data). Epidemics were observed in 1998 in Eritrea, Ethiopia, Turkey, Tunisia, and Morocco (Yahyaoui., 2004). During 2018 and 2019, the visits of farmers' fields in many regions of Morocco showed a low incidence of scald with fields showing medium severity including in mountainous and semi-arid regions (unpublished data). All released varieties in Morocco are moderately to highly susceptible to scald (Saidi et al., 2005), and host plant resistance was chosen as the most-appropriate approach for integrated management of the disease.

Our results showed that resistance was found in both FIGS and GCP subsets at the seedling and adult plant stages. The reaction to the disease differed between isolates, seasons, field locations, and between subsets at both stages. The differences in reactions could be attributed to virulence differences among the isolates at the seedling stage with isolate SC-511 being more aggressive than isolate SC-1122, and to differences in virulence spectra among scald field populations at the adult plant stage (Fig. 1). A similar finding was reported by Albustan et al in (1998). where they found 31% resistant genotypes at the adult plant stage and 19% at the seedling stage.

Although sources of resistance to scald were identified in both subsets at both stages, in general FIGS subset presented higher percentages of resistant accessions (54%) at both stages compared to GCP subset (45%). Likewise, 26% accessions in a Spanish barley core collection (Silvar et al., 2010), and 13% landraces from a set of lines derived from Syrian and Jordanian landraces (van Leur et al., 1989) were resistant to scald. Our study is the first attempt to compare FIGS to another subset, and the results are supporting the efficiency of this approach in mining genebank collections. Several other studies have shown the limitation of the core collection

approach for identifying and searching for rare and adaptive alleles (Dwivedi et al., 2008;Pessoa-Filho et al., 2010). In addition, the core collection and the GCP reference set derived from it are targeting higher overall diversity of the accessions, while FIGS is targeting the diversity for resistance to scald. However, more research is needed to assess the efficiency of both GCP and FIGS of finding different effective genes for adaptive traits such as resistance to scald. Furthermore, genotyping and association mapping studies could decipher if resistance is conditioned by new resistance genes or by new allelic variants of the existing resistance genes.

The high likelihood of containing the targeted alleles in FIGS-subset is most probably due to the co-evolutionary process that occurs over time between the host and the pathogen, and which might allow the maintenance of a high number of effective avirulence genes in the pathogen populations. Several studies demonstrated that the environment has a great influence on the development of the disease as well as the genetic structure of both the host and pathogen through processes of natural selection, gene flow, and spatial/geographic differentiation (Spieth., 1979 ; Epperson., 1990). The filtering approach of FIGS was based on environmental conditions best suited for the natural development of the disease and this approach might not be applicable to the favorable areas where improved varieties are used at a large scale, and which has affected the diversity of the pathogen populations by continuous selection of more virulent races. In addition, our results support to some extent the theoretical basis of FIGS approach and presents further evidence that the geographical distribution of the disease resistance in nature is not random, and it is influenced by environmental conditions and the host diversity. However, our FIGS modeling approach using machine learning did not show a strong relationship between resistance to scald and environmental condition for all isolates and field populations. This could be due to the limited size of the sets, and the unbalanced distribution of reaction to scald. In addition, this could also be a result of the variability within a collection site (van Leur et al., 1989), or a limited correlation between disease resistance and environmental conditions as Silvar et al in (2010) reported that resistance was found across the country. However, more resistance was found in winter types in Spain and at higher altitudes of Ethiopia. This geographic structure of disease resistance distribution was also evidenced for stem rust in wheat (Bonman et al., 2007), and powdery mildew in barley (Paillard et al., 2000).

Several previous studies using FIGS approach in different crops supported the evidence of identification of desirable expression traits using eco-geographical data (El Bouhssini et al., 2009, 2011, Bhullar et al., 2010b). FIGS approach was successfully used to identify source of resistance to stem rust and powdery mildew in wheat (Bari et al., 2014; Bhullar et al., 2009,

2010b), and net-blotch in barley (Endresen et al., 2011). The superiority of FIGS filtering approach was not confirmed at Holetta-nursery 2017 in Ethiopia, showing the need for refining the sub-setting process through consideration of the virulence spectra of the pathogen populations. The filtering approach can also be refined or improved further by the search of the best predictive models that can explain the relationship between the resistance and agro-climatic conditions using machine learning.

Using single isolates at the seedling stage, and the use of natural and artificial infection under the field conditions, the results showed that the reactions of both FIGS and GCP subsets to *R. commune* population are highly variable. The instability of responses could be attributed to the variable nature of the *R. commune* pathogen in terms of pathogenicity, genetic makeup of barley genotypes grown in a region, and response to environmental factors. The major resistance gene will exert selection pressure on virulence allele frequency of the pathogen isolates which will eventually break down the deployed resistance within a short span. Selection is one of the evolutionary factors which can be controlled to some extent by human interventions to slow down the evolution of genetic variants with altered pathogenicity. Mert and Karakaya in (2004) found 7 barley cultivars resistant to all five *R. commune* isolates from Turkey. In another study, (Azamparsa et al., 2015) treated a barley cultivar (Tokak 157/37) with gamma irradiation and tested 25 advanced mutant lines with three virulent isolates of scald from Turkey. Though Tokak 157/37 was highly susceptible to three *R. commune* isolates, two mutant lines were resistant to at least two isolates tested. This approach is suitable to introduce resistance in popular barley cultivars which are difficult to replace. Similar findings of high variability of the pathogenicity of the *R. commune* were also reported across the world (Avrova and Knogge., 2012; Azamparsa and Karakaya., 2020; Burdon et al., 1994). All studies suggested complex interactions of scald isolates with barley genotypes and the determination of pathotype diversity is essential to develop effective screening protocols.

The observed variability in virulence in *Rhynchosporium* is probably due to the high genetic variation of the pathogen population as reported by several studies, (Linde et al., 2009b; Mcdonald, 2015). The use of molecular markers showed limited genetic diversity in scald isolates from West Asia and North Africa, (Bouajila et al., 2010; Von Korff et al., 2005b), however, high genetic variation was reported from Australia, California, Finland, and Norway *R. commune* populations (McDonald et al., 1999a; Salamati et al., 2000). But a clear correlation between molecular markers and pathogenicity of scald populations is lacking which indicates the involvement of other molecular mechanisms which drive the evolution of virulence in *R. commune*. In general, virulence genes are located on chromosomal regions rich in transposable

elements which are more prone to recombination/mutation providing an evolutionary advantage to the pathogen to come up with novel variations in its effector repertoire to evade host defenses. The study of the evolution of necrosis-inducing protein (NIP1) effector from 146 strains of four *Rhynchosporium* spp. revealed its presence in all species, but presence/absence polymorphism in addition to copy number variation in *R. commune* revealed a strong selection pressure on this effector protein. The deployment of major resistance genes in rotation along with prudent use of fungicides can reduce the selection of virulent isolates and enhance the longevity of deployed resistant cultivars.

II.5. Conclusion

In the present chapter, our findings shows that the Focused Identification of Germplasm Strategy (FIGS) based on filtering agro-climatic conditions favoring the development of the disease has shown its efficiency in identifying a higher frequency of resistant accessions compared to the Reference Set of the Generation Challenge Program (GCP). However, more refinement of this approach is needed to take into consideration the specter of virulence of the pathogen and the development of best-bet models linking resistance to environmental conditions using machine learning.

Furthermore, this chapter showed that this research emphasizes the significance of the FIGS approach in identifying resistant traits and shed light on the intricate interplay between host genetics, pathogen diversity, and environmental conditions. Such insights are crucial for developing effective strategies to manage and mitigate the impact of scald and similar diseases on barley cultivation. Further exploration of the molecular mechanisms driving pathogen evolution and host-pathogen interactions could offer valuable directions for future research in enhancing crop resistance strategies. Ultimately, the findings presented in this research contribute not only to the advancement of barley breeding practices but also to the broader understanding of how machine learning can revolutionize crop improvement and secure global food production.

Chapter III: Identification of sources of resistance to scald (*Rhynchosporium commune*) and of related genomic regions using Genome-Wide Association in a mapping panel of spring barley

III.1 Introduction

Barley ranks the second most important temperate cereal crop in the world after *Triticum aestivum L*. Globally, it was sown over 51 million hectares with a production of 150 million metric tons in 2019 (FAOSTAT., 2020). Barley use is not limited only to feed, food and beverages, but several studies have highlighted it's nutritional value and health benefits including the potential for reducing colon cancer incidence, and lowering cholesterol (FDA., 2006; Kawka et al., 2019; D. Li et al., 2021).

The quality and productivity of barley crop are affected by abiotic and biotic stress factors. Among biotic stresses, leaf blotch or scald caused by the hemibiotrophic ascomycete fungus *Rhynchosporium commune* (formerly known as *R. secalis*) is one of the most economically important and destructive disease of barley worldwide, which can reduce grain quality and inflict high yield losses (Perfect & Green., 2001; Xi et al., 2000; Zhan et al., 2008). *Rhynchosporium commune* is known for its high genetic diversity which allow the pathogen population to overcome resistant cultivars, and to develop resistance to fungicides within a short period of time (Brunner et al., 2016; Mcdonald., 2015; Mohd-Assaad et al., 2016; Stefansson et al., 2012, 2013). The inherent complexity of the fungus requires an integrated disease management approach (McLean & Hollaway, 2018; Stefansson et al., 2012), and genetic resistance is the most economical, sustainable, and environment friendly strategy for controlling barley scald disease.

Development and deployment of resistant barley cultivars through incorporation and pyramiding of both major and minor genes can provide durable resistance against *R. commune* (Walters et al., 2012). Scald disease resistance can be classified as horizontal or vertical, both types of resistance are based on the number of "*R* genes" involved. Vertical resistance is qualitative in nature while horizontal resistance is governed by minor genes.

However, both vertical and horizontal resistance mechanisms are used by breeders. Compared to other barley diseases, relatively a few resistance loci against *R. commune* have been discovered which can be employed in breeding programs (Dracatos et al., 2019; Clare et al., 2020; Zhang et al., 2020). The qualitative resistance genes, frequently detected using specific strains at the seedling stage, provide a high level of resistance at all growth stages (Zhan et al., 2008). However, quantitative resistance *to R. commune* identified at the adult plant stage

provides a partial level of resistance (Wallwork and Grcic., 2011, Zhan et al., 2008). The first *R. commune* resistance gene was designated as *Rrs1* which conferred resistance under field conditions, and under controlled conditions with specific isolates (Looseley et al., 2018; Zhan et al., 2008a). Frequently, barley scald disease resistance loci were detected on chromosome 3H flanking *Rrs1* locus, and on chromosome 7H flanking *Rrs2* locus (Looseley et al., 2018; Zhan et al., 2008a). Across the barley genome, several major and minor *R* genes, and QTLs for leaf scald resistance have been identified in different barley genotypes scattered across the seven barley chromosomes (Zhang et al., 2020). To date, 11 major loci including *Rrs1*, *Rrs2*, *Rrs4*, *Rrs12*, *Rrs13*, *Rrs14*, *Rrs15*, *Rrs16*, *Rrs17*, (*Rrs15* (CI8288), and *Rrs18* controlling resistance to *R. commune* have been identified. Further, the major scald resistance genes have been detected on all chromosomes except for 5H, with *Rrs14* on chromosome 1H, *Rrs17* on chromosome 2H, *Rrs1* and *Rrs4* on chromosome 3H, *Rrs3* and *Rrs16* on chromosome 4H, *Rrs13* and *Rrs18* on chromosome 6H, and *Rrs2*, *Rrs12* and *Rrs15* on chromosome 7H (Bjørnstad et al., 2002; Zhang et al., 2020). Most of the modern barley cultivars share the same allele combinations of resistant loci indicating the limited genetic diversity among the barley germplasm (Williams et al., 2003b). Furthermore, owing to the complex virulence spectrum and ability of *R. commune* populations to overcome resistant cultivars, durable resistance against scald disease requires continuous research for polygenic resistance (Xi et al., 2000).

Breeders and researchers are using landraces and wild relatives genebank accessions, panels and genetic stocks to identify new sources of resistance. There are various sources of resistance derived from *Hordeum vulgare* ssp. *vulgare* (Hofmann et al., 2013; Silvar et al., 2010), from wild barley *Hordeum vulgare* ssp. *spontaneum* (Von Korff et al., 2005b; Yun et al., 2005) and from *Hordeum bulbosum* (Pickering et al., 2006).

In recent years, with the availability of a high-quality reference genome assembly (Mascher et al., 2017), and of high-density genotyping platforms (Comadran et al., 2012b; Moragues et al., 2010), there has been a growing interest in genomic resources of barley. Different statistical methods have been developed and used to identify molecular markers that are linked to traits of interest in bi-parental QTL mapping (linkage mapping) and in genome wide association mapping (Linkage dis-equilibrium mapping). The allelic richness and the genetic mapping resolution in bi-parental mapping populations are mainly affected by the genetic recombination and segregation (Alqudah et al., 2020). Genome-wide association studies (GWAS) have proven to be an efficient tool that captures greater genetic variation and offers high resolution mapping of QTL and associated candidate genes compared to classical linkage analysis (Bayer et al., 2017; Cockram et al., 2008; Comadran et al., 2012b). In barley, GWAS has been successfully

applied to identify QTLs associated with resistance to net blotch (Amezrou et al., 2018), powdery mildew (Czembor et al., 2022), spot blotch (Visioni et al., 2020), and barley stripe rust (Visioni et al., 2018).

The main objectives of this study were to identify sources of resistance to scald in a diverse panel of barley genotypes at the seedling and at the adult plant stages, and to use GWAS to identify the underlying genomic regions conditioning resistance to *R. commune*.

III.2 Materials and methods

III.2.1 Plant material

A collection of 316 spring barley genotypes designated as association mapping-panel 2017 (AM2017) constructed by barley breeders at the International Center for Agriculture Research in the Dry Areas (ICARDA) was used in this study. It has 143 six-row and 173 two-row type barley genotypes, consisting of 134 advanced breeding lines from ICARDA, 21 landraces from ICARDA genebank, 161 released cultivars from Asia, Africa, Europe, and America (Verma et al., 2021).

III.2.2 Seedling stage disease resistance test (SRT)

Scald infected barley leaves were collected from naturally infected barley fields from different agro-ecological zones of Morocco during the disease surveys conducted from 2015 to 2018. Isolation of *R. commune* was performed by soaking the scald infected barley leaves in sterile distilled water for 15 min followed by surface sterilization using 10% sodium hypochlorite solution for 30 s and 50% ethanol for 15 s, rinsed with sterile distilled water three times, and dried up within two layers of sterile Whatman filter paper. The leaf segments were incubated at 14 °C in darkness for two weeks on Lima Bean agar (LMA) supplemented with Kanamycin and Streptomycin (50 mg per liter). After two weeks of incubation, the sporulating white or pinkish colonies were streaked on new LBA plates for the preparation of single conidial isolates.

Four seeds of each genotype were planted in a single cone of 3.8 cm diameter and 14 cm depth containing peat moss supplemented with 14-14-14 NPK fertilizer in 98-Ray Leach containers (Stuewe & Sons, Inc., Oregon, USA) in 3 replications. Seedlings of the AM2017 were grown under controlled conditions with a photoperiod of 16 h light/8 h dark at 20 ± 1 ^{o}C in the growth chamber (Model MC1750; Snijder Scientific, Tilberg, Netherlands) at ICARDA, Rabat, Morocco. Three virulent isolates of *R.* commune, SC-511, SC-1122 and SC-611 collected from Marchouch (MCH), Sidi Allal Tazi (SAT), and Annoceur (ACR) were used for seedling

resistance. The inoculum was prepared from the 10-12 days old LBA plates by rubbing the agar surface with sterile glass slide followed by filtration through double layer of cheese cloth. The inoculation of about 2 weeks old seedlings, with the second leaf fully extended, was performed using spore suspension of 5×10^5 conidia/ml supplemented with surfactant Tween 20 (0.01%) followed by incubation at 100% relative humidity in dark for 72 h at 15 °C in the growth chamber. Then the seedlings were transferred to a greenhouse with day/night temperature regime of 20 °C/16 °C with a photoperiod of 16h light and 8h of darkness. After 16 days post infection (dpi), the infection types were recorded using disease rating scale of 0 to 5 (Salamati & Tronsmo., 1997) where 0 = I (Immune), 1= R (resistant), 2 = MR (moderately resistant), 3 = MS (moderately susceptible), 4 = S (susceptible), and 5 = HS (highly susceptible).

We also checked the virulence spectrum of three *Rhynchosporium commune* (*Rc*) isolates SC-1122, SC-511, and SC-611 on nine barley differential Atlas, Atlas 46, Brier, CI3515, CI4364, Jet, La Masita, Osiris, and Turk. These differentials possess specific resistance genes (R-genes) and have been previously characterized for their response to *Rc* isolates. In addition, barley differential interactions were examined to determine the compatibility between the *Rc* isolates and the different host genotypes. The presence or absence of compatible interactions, indicated by disease development, and incompatible interactions, indicated by the absence of disease symptoms, were recorded for each combination.

III.2.3 Adult plant stage disease assessment (APS)

Field trials were conducted in Morocco at the INRA experimental stations of Marchouch (MCH; 33° 56 N, 6° 63 W) and Guich (33° 58 N, 6° 51 W), and at Rommani region during the cropping season of 2018-2019. Each entry was planted as paired row of 1meter length with row spacing of 0.5 m in an augmented design using two susceptible (Tissa and Tocada) and two resistant checks (Atlas 46 and ICARDA 4). To ensure high disease pressure, each block was surrounded by a border composed of a mixture of scald susceptible genotypes (Tissa, Aglou, Tiddas, Fleet, Adrar, Shepherd, Baudin and Alester).

Starting from Zadoks scale GS30 (Zadoks et al., 1974) at an interval of 10-12 days, the trials were artificially inoculated four times using a knapsack sprayer with inoculum composed of a mixture of 15 isolates of *R. commune* which were collected from different agro-ecological zones of Morocco. Furthermore, scald infected barley residues from the previous growing season were uniformly distributed over the trial to ensure disease establishment. In addition, the development and spread of the disease was favoured by applying frequent mist irrigation in late

afternoons daily. The disease severity was assessed at GS 73-75 using 0-9 scale (Saari & Prescott, 1975), and the genotypes were categorized into five classes; 0 to 2 as resistant, 3 to 4 as moderately resistant, 5 to 6 as moderately susceptible, 7 to 8 as susceptible and 9 as highly susceptible. The area under the disease progression curve (AUDPC) was calculated using the following equation (Jeger and Viljanen-Rollinson, 2001)

$$AUDPC = \sum_{\alpha}^{1} i\, 1^{n}[RC_{I+1} + RC_i/2][t_{i+1} - t_i]$$

Where RC_i = *R. commune* severity on *i*th days, t_i = time in days at *i*th observation, and *n* is the total number of observations.

III.2.4 Data analysis

The frequencies of different classes of reaction to *R. commune* at the seedling and adult plant stages were performed using R software (R Core Team., 2019) and the Venn diagrams were developed to show the number of resistant entries common between the three isolates and among the three fields. To gain a comprehensive understanding of the genetic control of scald resistance and its potential for improvement through breeding efforts, narrow-sense heritability (H^2) of scald genetic resistance was estimated for each test at the seedling and adult plant stages using the variance component method. The heritability estimate was calculated as the ratio of additive genetic variance to the total phenotypic variance (Falconer and Mackay., 1996) with the use of R software.

III.2.5 Genotyping, population structure, and linkage disequilibrium of AM2017

Genomic DNA extraction, genotyping, population structure, and genetic diversity of the 316 genotypes used in this study has been described in an earlier study by Verma et al. (2020). Isolation of genomic DNA was conducted at the growth stage (GS12) using a lyophilized young leaf tissue from a single plant at the Cereal Crop Research Unit, USDA-ARS, Fargo, North Dakota, USA (Slotta et al., 2008). The Illumina iSelect 50K SNP array for barley (Illumina, San Diego, CA, USA) was used to perform SNP genotyping following the manufacturer's guidelines (Bayer et al., 2017). Further, 36,793 SNP markers were used for genetic analysis after quality control by discarding all monomorphic markers, markers with missing values of more than 20 %, and with minor allele frequency (MAF) of less than 5%.

The population structure of AM2017, was assessed using the filtered SNP markers to estimate individual admixture coefficients and was performed using the sparse Non-negative Matrix

Factorization (sNMF) algorithm implemented in the R package *LEA* (Frichot & François, 2015). The cross-entropy criterion was used from sNMF function to evaluate several putative populations (K) ranging from K=2 to K=10 (Alexander & Lange., 2011; Frichot et al., 2014). For each K we set the number of iterations to 200, the number of runs to 10, alpha to 10, and tolerance to 10^{-5}. The genotypes declared admixed or were assigned to subgroups using 80% membership criterion. The Principal Component Analysis (PCA) was performed with the 36,793 filtered SNP markers using TASSEL version 5.0. Furthermore, the kinship matrix (K) among 316 genotypes was computed using filtered set of SNP markers in TASSEL 5.0 (Bradbury et al., 2007).

The linkage disequilibrium (LD) was determined for all pairs of loci using SNP markers with known positions. The squared allele-frequency correlations (r^2) were computed in PLINK V1.9 using non-linear regression with a threshold set at 0.2. The extent of genome wide LD decay was visualized by plotting intra-chromosomal r^2 values against the physical distance in cM (Purcell et al., 2007).

III.2.6 Genome-wide association study (GWAS)

The association mapping was conducted in Tassel version 5.2.53 software by combining phenotypic and genotypic data (Bradbury et al., 2007b; Lipka et al., 2012). Two general linear models (GLM+Q), and (GLM+ PCA), and two mixed linear models, (MLM+Q+K), and (MLM+PCA+K), were tested by taking into account kinship (K matrix), population structure (Q matrix), and principal coordinate analysis (PCA) as covariates to control false positives. In addition, several GAPIT version 3.0 models (Lipka et al., 2012) were used to validate significant SNP markers/loci associated to scald resistance. In GAPIT3, GWAS was performed using Settlement of MLM Under Progressively Exclusive Relationship (SUPER) (Wang et al., 2014), Multiple-locus MLM (MLMM) (Segura et al., 2012), Fixed and random model Circulating Probability Unification (FarmCPU) (Zhang et al., 2010), and Bayesian-information and Linkage-disequilibrium Iteratively Nested Keyway (BLINK) (Huang et al., 2019).

A GWAS threshold P-value of $< 2.0 \times 10^{-4}$ [$-\log10(\text{P value}) < 3.7$] was used for declaring significant-marker trait associations as reported by Borrego-Benjumea et al., (2021). This hybrid approach was based on the median of two threshold methods: a stringent method proposed by Wang et al., (2012) determines the significance threshold using the equation $\alpha = 1/m$, where 'm' represents the number of markers. In this case, $-\log_{10}(\text{P-value}) < 4.4$ was considered significant; and a less stringent approach (Chan et al., 2010) employs the use of

bottom 0.1 percentile of P-values distribution [−log10(P value) < 3.0]. It balances the need for stringent control of Type I error (false positives) with the goal of discovering meaningful associations. In addition, we also used LD adjusted Bonferroni correction (Dugal et al., 2008; Visioni et al., 2020) which declared the P-value significance threshold to < 2.0×10^{-4} [−log10(P value) < 3.7]. Furthermore, QTL being validated by our study were also kept with a cut-off value of [−log10(P value) < 3.0].

The additive genetic effect refers to the portion of phenotypic variation that can be attributed to the additive effects of multiple genetic loci. In the output, R^2 value explains how much of the phenotypic variation can be accounted for by the significant SNP marker. A higher R^2 value indicates a stronger relationship between the marker and the observed trait. While the positive or negative allele effect indicates whether the allele increases susceptibility or provides resistance to the disease, respectively. When the allele effect is positive, it suggests that possessing that allele increases the disease score, indicating susceptibility to the disease. Conversely, a negative allele effect signifies that the allele reduces the disease score, indicating resistance to the disease. The Manhattan plots of GWAS were generated using CMplot package in R 3.3.1.

III.2.7 QTL alignment and candidate genes

The marker sequences of previously reported QTL were retrieved from Grain Genes database (https://wheat.pw.usda.gov/blast/), and their positions were checked on the barley pseudomolecules Morex v. 2.0 2019 and Morex v3 pseudomolecules (2021) using Barleymap pipeline (Cantalapiedra et al., 2015). For candidate genes (CGs), the sequences of significant SNP markers were subject to BLAST search tool of IPK barley server (https://galaxy-web.ipk-gatersleben.de/) and based on a threshold of BIT score (> 200), sequence identity (90-100%), and an expect value ($0 – 1^{\cdot 40}$). The candidate gene search considered the presence of proteins with functional domains implicated in plant disease resistance.

III.3 Results

III.3.1 Seedling resistance of AM2017 panel with three isolates of *R. commune*

Analyses of the phenotypic data showed a uniform disease establishment and diverse infection responses (IR) depending on the virulence spectrum of each isolate at the seedling stage under controlled conditions. The average IR was 0 and 5 respectively for the resistant (ICARDA 4 and Atlas 46) and susceptible (Tocada and Tissa) checks for all the three isolates.

An average IR of 1.52 for *Rc* isolate SC-S6, 1.98 for isolate SC-S511, and 3.6 for isolate SC-1122 was observed in the AM2017 panel. Moreover, the frequency distribution of IR of AM2017 panel followed a normal distribution for the *Rc* isolates SC-511 and SC-611, whereas the distribution was negatively skewed towards susceptibility for the isolate SC-1122 (Figure III.1). Furthermore, the highest percentages of resistant genotypes were observed for the *Rc* isolate SC-S6 with 19.1% (60) immune and 30.3% (95) resistant genotypes, followed by the *Rc* isolate SC-511 with 6.7% (22) immune and 23.4% (74) resistant genotypes, whereas the *Rc* isolate SC-1122 showed the highest cumulative percentage of 73.8% (233) of susceptible and highly susceptible genotypes (Figure III.1). The *Rc* isolate SC-511 showed the highest percentage of moderately resistant (23.2%) genotypes followed by SC-S611 and SC-1122 isolate with 23.2% and 4.4%, respectively. Six-row genotypes had higher percentage of resistant barley genotypes with an average IR of 1.25 ± 0.9 for *Rc* isolate SC-511, followed by IR of 1.4 ± 1.1 for SC-S6, and IR of 2.9 ± 1.5 for SC-1122, whereas, the two-row barley genotypes were prone to susceptibility with an average IR of 1.62 ± 1.3 for SC-S6, followed by 2.6 ± 1.1 for SC-S511, and 4.1 ± 1.2 for SC-1122 isolates. About 11 barley genotypes, AM5, AM19, AM39, AM51, AM53, AM59, AM165, AM221, AM248, AM267, and AM280 were resistant to all the three *Rc* isolates used (Figure III.2). The heritabilities of scald resistance for all the three isolates at the seedling stage ranged from 0.40 to 0.58 (Annex III.1).

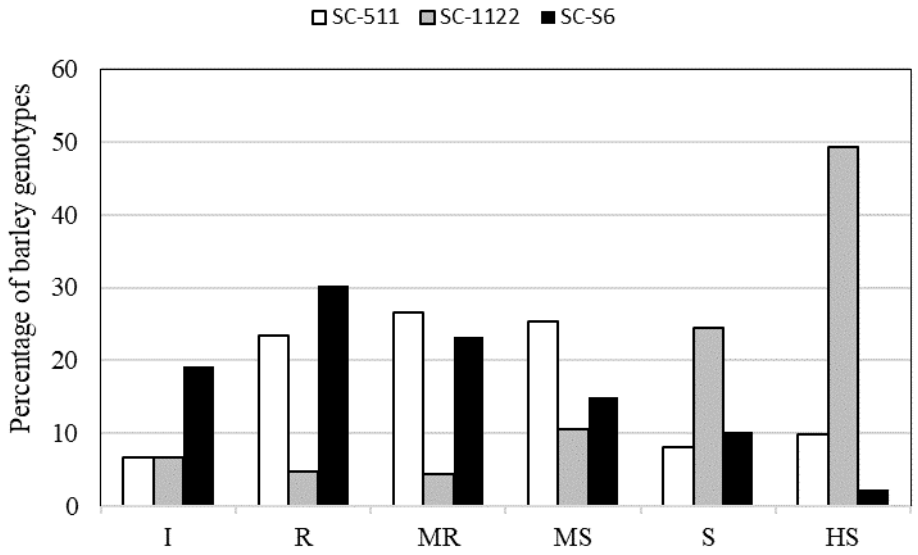


Figure III.1. Frequency distribution of reaction of 316 barley genotypes of AM2017 mapping panel at the seedling stage against *R. commune* isolates, SC-511, SC-611, and SC-1122.

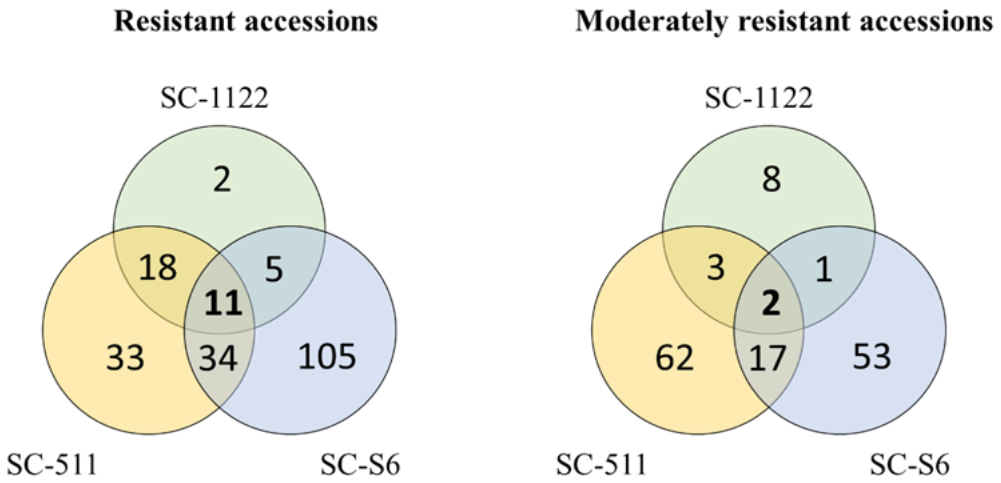


Figure III. 2. Venn diagrams showing barley genotypes with combined resistance at the seedling stage to the three *R. commune* isolates under controlled conditions

The interaction between barley differentials against the three *Rc* isolates showed that the *Rc* isolate SC-1122 was avirulent on Atlas (*Rrs2*), Atlas 46 (*Rrs1, Rrs2*), Brier (*Rrs1*), however it was virulent on CI3515 (*Rh4, Rh10*), and La Masita (*Rh10, Rh4*). The *Rc* isolate SC-511 was virulent on Atlas (*Rrs2*), Brier (*Rrs1*), CI4364 (*rh11*), and CI3515 (*Rh4, Rh10*), but it was avirulent on Atlas 46 (*Rrs1, Rrs2*), Jet (*Rh6, rh7, rh6*), Osiris (*Rh4, rh6, Rh10, BRR6*), and Turk (*Rh5, Rrs1*$_{Turk}$*, rh6*). However, the *Rc* isolate SC-611 was avirulent on all of the differentials tested except Skiff. A differential interaction was observed for the *Rc* isolate SC-511 on Atlas, Brier and CI4364. It can be concluded that the *Rc* isolate SC-511 may be NIP1 deficient as it displayed compatible interaction on Brier (*Rrs1*) as reported previously (Mohd-Assaad et al., 2019; T. Stefansson et al., 2014). Interestingly, it also showed compatible interaction on Atlas (*Rrs2*). Furthermore, four differentials showed incompatible interaction against the three *Rc* isolates tested; Atlas 46 (*Rrs1, Rrs2*), Jet (*Rh6, rh7, rh, rh6*), Osiris (*Rh4, rh6, Rh10, BRR6*), and Turk (*Rh5, Rrs1Turk (Rh3), rh6*). A differential interaction was observed for the *Rc* isolate SC-511 on Atlas, Brier and CI4364. Furthermore, four differentials showed incompatible interaction against the three *Rc* isolates tested; Atlas 46 (*Rrs1, Rrs2*), Jet (*Rh6, rh7, rh, rh6*), Osiris (*Rh4, rh6, Rh10, BRR6*), and Turk (*Rh5, Rrs1Turk (Rh3), rh6*).

III.3.2 Adult plant stage phenotyping of AM2017 panel

At the adult plant stage, the disease severity on barley genotypes differed based on the environmental conditions in each location and the local pathogen population (Figure III.3). At Marchouch (MCH18) and Rommani (AR18) in 2018, the average disease severity of 1 on the resistant (ICARDA4 and Atlas 46) and 9 on the susceptible (Tocada and Tissa) checks were

observed. While at Guich in 2018 (Guich18), the average disease severity of 0 and 7 was observed on the resistant and susceptible checks respectively.

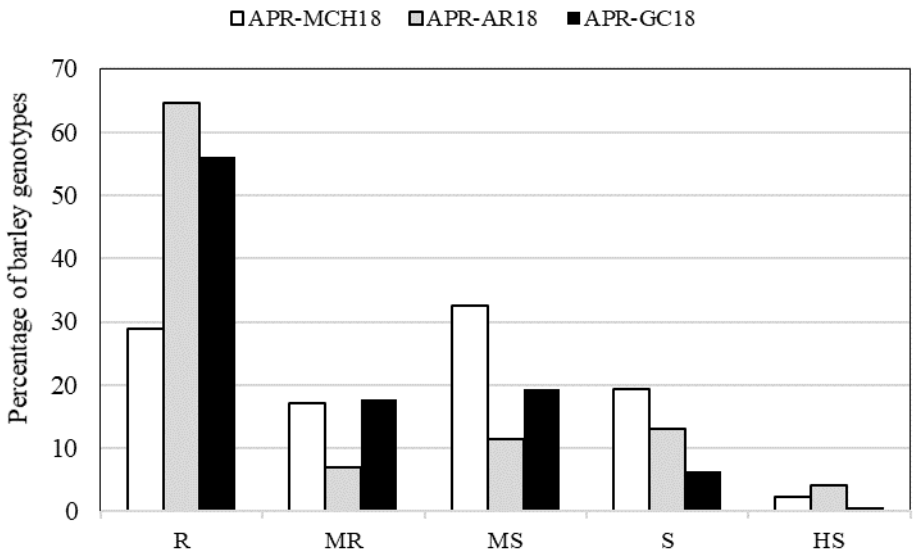


Figure III.3. Frequency distribution of leaf scald resistance in 316 barley genotypes of AM2017 mapping panel at the adult plant stage at Marchouch (MCH18), Rommani (AR18), and Guich (Guich18)

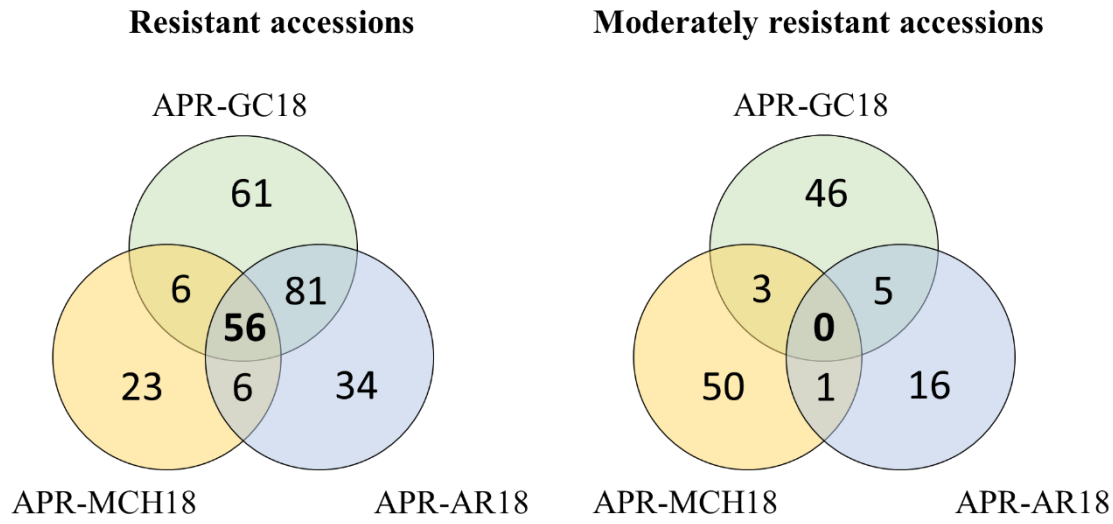


Figure III.4. Venn diagrams showing barley genotypes with combined resistance at the adult plant stage at three field locations

The highest percentage of scald resistant genotypes was observed at AR18 with 64.5 % (204) of genotypes compared to 56% (177) at Guich18 and 28.8% (91) of resistant genotypes at MCH18 (Figure III.3). Likewise, the highest percentage of scald susceptible genotypes were found at MCH18 with 21.5 % (68) of genotypes, followed by 17.1% (54) genotypes at AR18, and 6.9% (22) susceptible genotypes at Guich18. About 56 genotypes were resistant across

locations, whereas only seven genotypes displayed combined resistance at the seedling and adult plant stages (Figure III.4, Table III.1).

Table III.1: Immune (I) and resistant (R) barley genotypes in AM2017 mapping panel to three *R. commune* isolates at the seedling stage and at the adult plant stage at three field locations in Morocco.

Line #	Pedigree	Row type	SRT-SC511	SRT-SC1122	SRT-SCS6	APR-AR18	APR-Guich18	APR-MCH18
AM5	ICARDA SN326	6	I	I	I	R	I	R
AM39	M104/PFC 88210//DOÑA JOSEFA	6	I	R	I	R	R	R
AM51	HB511/JAEGER H00056005 09/3H0078	6	R	I	I	R	I	R
AM53	P.STO/3/LBIRAN/UNA80//LIGNEE640/4/BLLU/5/PETUNIA 1/6/CHAMICO/TOCTE//CONGONA	6	I	R	I	R	R	R
AM59	MSEL/FNC1	2	R	I	I	R	I	R
AM248	Isaria	6	I	I	R	R	I	R
AM280	15UCM 92	2	I	I	I	R	I	R

III.3.3 Population Structure and linkage disequilibrium

We performed principal component analysis (PCA) using a filtered set of 36,793 SNP markers to evaluate the genetic diversity of the 316 barley genotypes. The PCA of AM2017 categorized the panel into distinct clusters and showed a clear differentiation based on row type and germplasm type. About 10.6 % of the genotypic variance was explained in the first axis of the PCA, partitioned the genotypes into two clearly defined groups according to their row type, being the six-rowed genotypes located towards in the positive side of the axis and the two-rowed genotypes in the negative side (Figure III.1.). The same separation was observed using sNMF, cross-validation and the cross-entropy criterion approaches, indicating that the AM2017 panel at K=2 is partitioned into two major clusters based of row types (Figure III.1.).

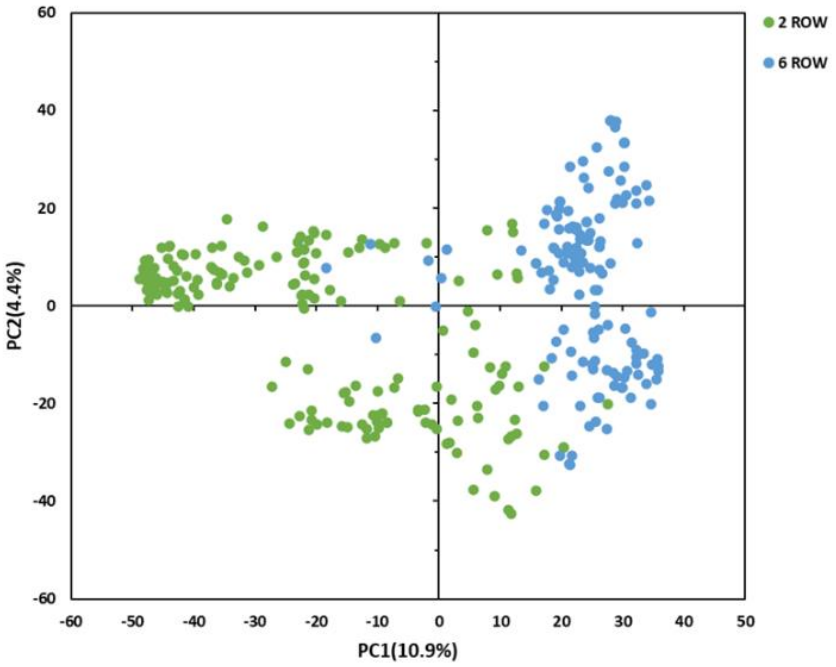


Figure III. 5. Principal component analysis (PCA) using 36,793 SNP markers displaying the spatial distribution of the 316 genotypes based on their row type

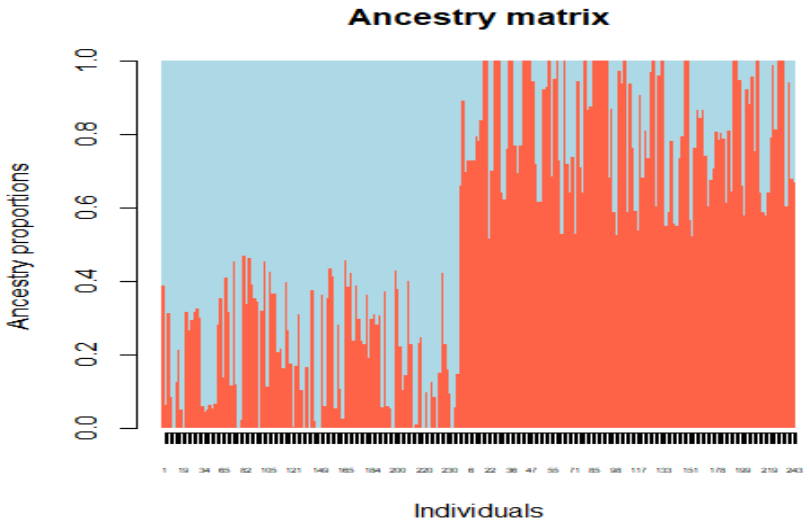


Figure III. 6. Population structure of AM17 using sNMF algorithm

About 40.18% of the two-row genotypes were represented by one cluster and 59.81% of the six-row genotypes were represented by the second cluster. In addition, the PCA of the AM2017 showed a separation according to the germplasm type, but the barley landraces were not grouped into distinct group (Figure III.3). The cross-entropy criterion did not show a clear plateau and steadily decreased at higher K values. However, significant decreases in the criterion were observed from 2 to 10 (Figure III.4). Furthermore, the LD decay of AM2017 was estimated to be ~600 kb (~0.4 cM) (r^2 = 0.2; Figure III.5).

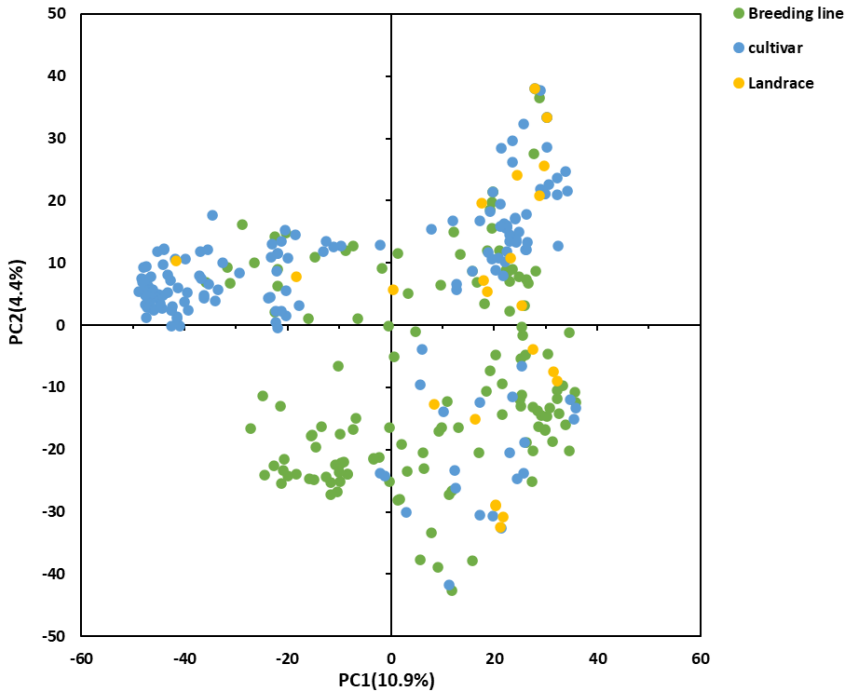


Figure III.7. Principal component analysis (PCA) using 36,793 SNP markers displaying the spatial distribution of the 316 genotypes based on their germplasm types

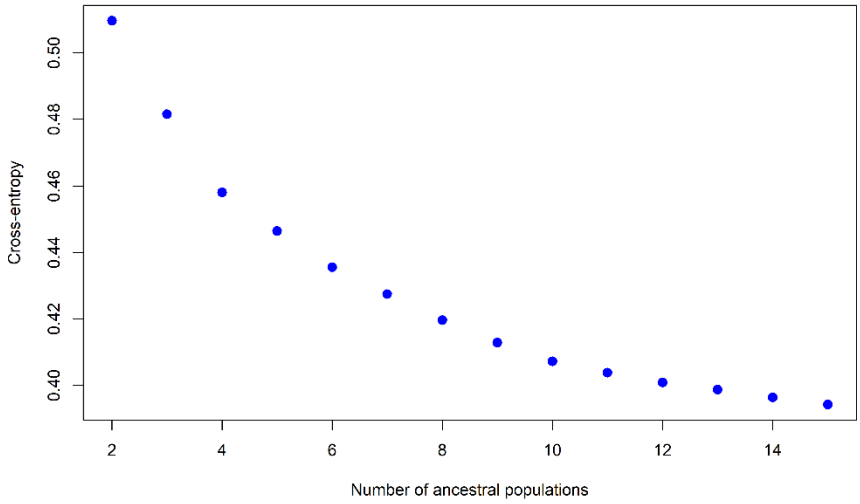


Figure III.8. Values of the cross-entropy criterion for sNMF algorithm to evaluate several putative populations (K) ranging from K=2 to K=10

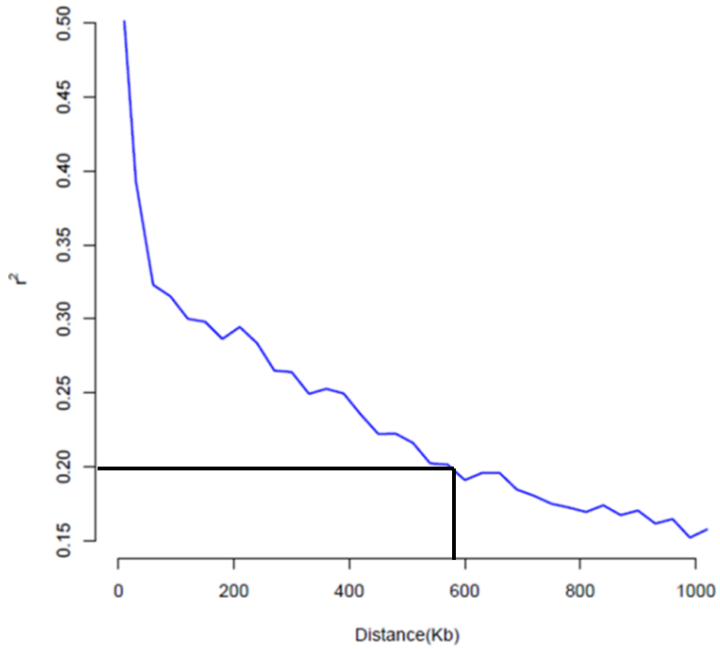


Figure III.9. the LD decay of association mapping panel 2017 using genetic distance

III.3.4 GWAS of scald resistance at the seedling stage

Performing GWAS for the assessment of resistance to the three *R. commune* isolates, SC-511, SC-1122 and SC-611 at the seedling stage, the best fitting model was the MLM procedure using PCA, accounting for population structure (Q) and relatedness (K) (**Figure III.6**). Our genome scan detected 102 significant MTAs corresponding to 15 QTL against the three *Rc* isolates at the seedling stage, with a range of marker R^2 from 3.55 – 9.84%, and additive effects ranging from -1.62 to 1.45 (Annex III.2). In addition, 14 (93%) out of 15, and 6 (50%) out of 12 APR QTL could be validated by more than 3 models. The genome scan for *Rc* isolate SC-511 detected 15 MTA corresponding to 5 QTL on chromosomes 2H and 7H, with marker R^2 and the additive effect ranging from 3.55 to 6.64 %, and from -1.02 to 0.905, respectively. The highest phenotypic variation of 6.64% was explained by the SNP marker JHI-Hv50k-2016-483776 associated with *qSc.SRT4* on 7H (390.164 Mb; 70.54 cM), reducing the disease severity by -0.886 (17.2%), and by the SNP marker SCRI_RS_226361 associated with qSc.SRT3 on 7H (5.168 Mb; 1.91 cM), reducing the disease severity by -0.637 (21%) and explaining 4.69 % of phenotypic variation.

For *Rc* isolate SC-1122, 55 MTAs corresponding to 7 QTL, were detected on chromosomes 3H and 7H, with marker R^2 and the additive effect ranging from 4.47 to 9.94 %, and from -1.62 to 1.45, respectively. The highest phenotypic variation of 9.94 % was explained by the SNP marker JHI-Hv50k-2016-182733 associated with *qSC.SRT10* on 3H (438.945 Mb; 51.63 cM),

reducing the disease severity by -1.62 (32.4%). In addition, the results demonstrated that several genomic regions were mapped at the vicinity of the previously mapped alleles loci on chromosome 3H (Figure III.7).

Likewise, for *Rc* isolate SC-611, 32 MTAs represented by 3 QTL were detected on chromosomes 2H and 7H, with marker R^2 and the additive effect ranging from 3.64 to 7.05 %, and from -0.88 to -0.775, respectively (Annex III.2). The highest phenotypic variation of 7.05 % was explained by a SNP marker JHI-Hv50k-2016-490465 associated with qSc.SRT15 on 7H (508.91 Mb; 77.41 cM). However, the highest reduction in disease severity of -0.88 (17.6%) was caused by the SNP marker JHI-Hv50k-2016-443516 associated with *qSc.SRT14* on 7H (7.666 Mb; 3.97 cM).

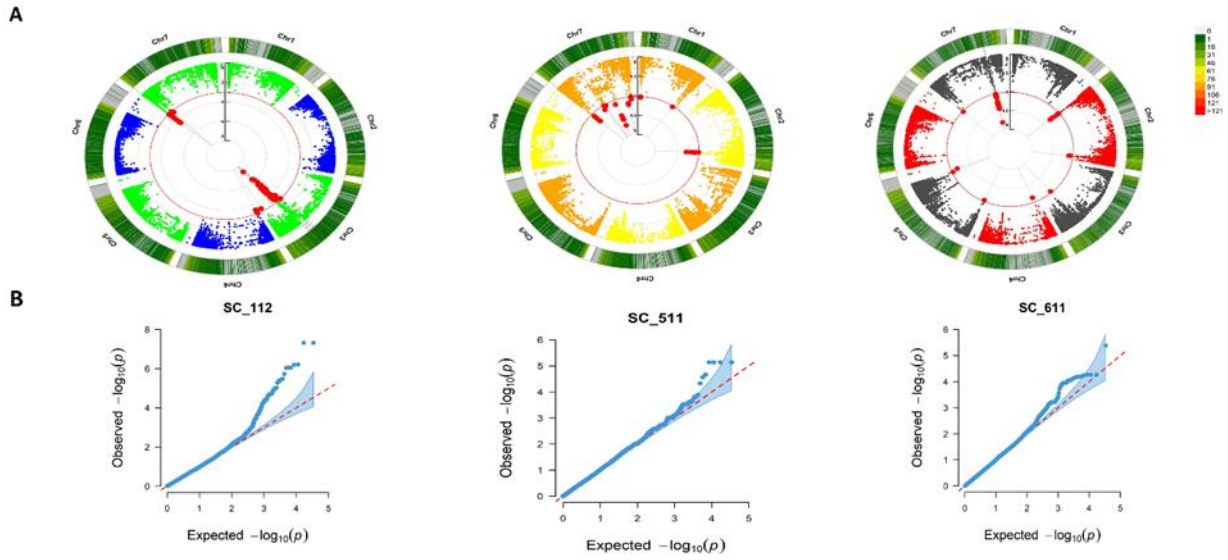


Figure III.10. Genome-wide association mapping of barley scald resistance at the seedling and adult plant stages. The Manhattan plots shows –log10 of p-values from genome-wide association mapping against the positions of SNPs on all chromosomes of barley at the seedling stage for *R. commune* isolate SC-511, SC-1122, and SC-S6 (A). Quantile-Quantile (Q-Q) plots of marker-trait association at the seedling stage at the seedling MLM model (B).

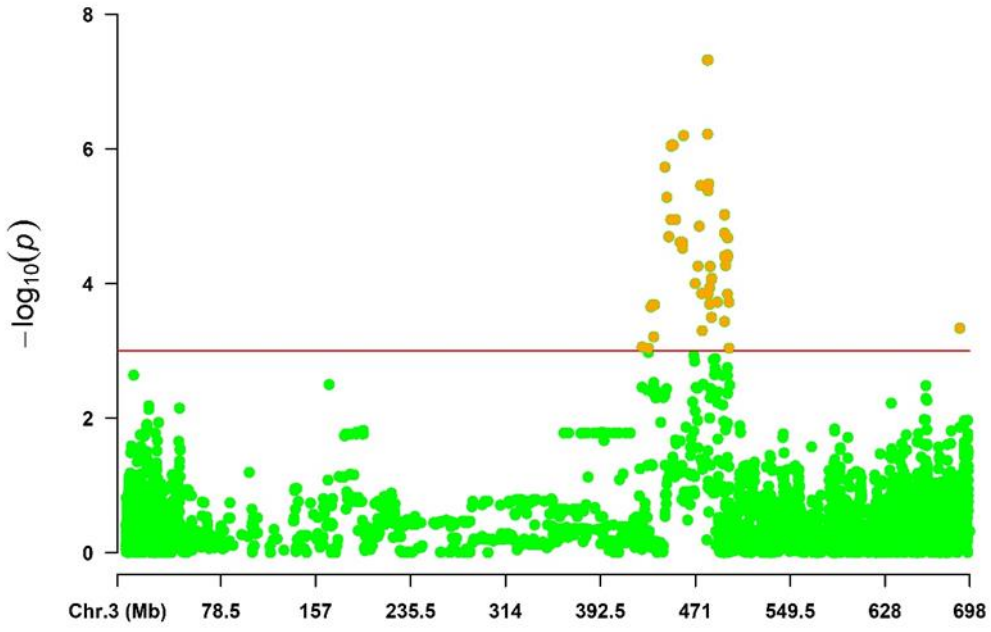


Figure III.11. Significant SNPs markers distributed across the chromosome 3H from the region 426 to 689 Mb associated with resistance to scald in the AM17 barley panel

III.3.5 GWAS of scald resistance at the adult stage

For the resistance at the adult plant stage, MLM procedure accounting for population structure (Q or PCA) and relatedness (K) was identified as the best fitting model (Q-Q plots for all tested models are shown in Figure 5). Our genome scan detected 25 significant MTA corresponding to 12 QTL scattered on all barley chromosomes except the chromosome 2H, with a marker R^2 ranging from 4.10 – 6.42%, and additive effects from -2.25 to -1.070 (Annex III.3). In addition, 19 MTAs, corresponding to 16 QTL with a LOD score of 3.0 – 3.6 have been presented (Annex III.3) as they had been reported in previous studies, thus validating our analysis.

In case of Rommani site (AR18), 9 MTA represented by 4 QTL on the chromosomes 1H, 4H, and 6H were involved in scald disease resistance with a range of marker R^2 of 4.55 to 6.42, and additive effect of -2.25 to -1.107. The highest phenotypic variation of 6.42% was explained by a SNP marker SCRI_RS_179398 associated with *qSc.APR11* on 4H (606.21 Mb; 103.9 cM), reducing the disease severity by -1.28 (14.2%). However, the highest reduction in disease severity of -2.25 (25%) was caused by a SNP marker JHI-Hv50k-2016-4445 associated with *qSc.APR9* on 1H (5.04 Mb; 4.96 cM), explaining a phenotypic variation of 5.7%. For Guich (Guich18), 11 MTA represented by g6 QTL were detected on all barley chromosomes except 2H and 6H with a range of marker R^2 of 4.10 to 5.63, and additive effect of -1.903 to -1.262 (Annex III.3). The highest phenotypic variation of 5.63% was explained by the SNP marker

JHI-Hv50k-2016-265990 associated with *qSc.APR6* on 4H (602.22 Mb; 99.43 cM), reducing the disease severity by -1.42 (15.7%). However, the highest reduction in disease severity of -1.9 (21.1%) was attributed to the SNP marker JHI-Hv50k-2016-469294 associated with *qSc.APR8* on 7H (75.12 Mb; 61.47 cM), explaining a phenotypic variation of 5.06%. For Marchouch (MCH18), 5 MTA represented by 2 QTL were detected on the chromosomes 4H and 7H with a range of marker R^2 4.51 to 5.58 and additive effects from -9.03 to -7.22. The highest phenotypic variation of 5.58% was caused by the SNP marker JHI-Hv50k-2016-438638 associated with *qSc.APR2* on 7H (3.376 Mb; 1.63 cM). However, the highest reduction in disease severity of -9.03 was caused by the SNP marker JHI-Hv50k-2016-440870 associated with *qSc.APR2* on 7H (4.83 Mb; 52.01 cM). Interestingly, 11 SNP markers were associated with *Rc* resistance at both the seedling and the adult plant stages (Table III.2).

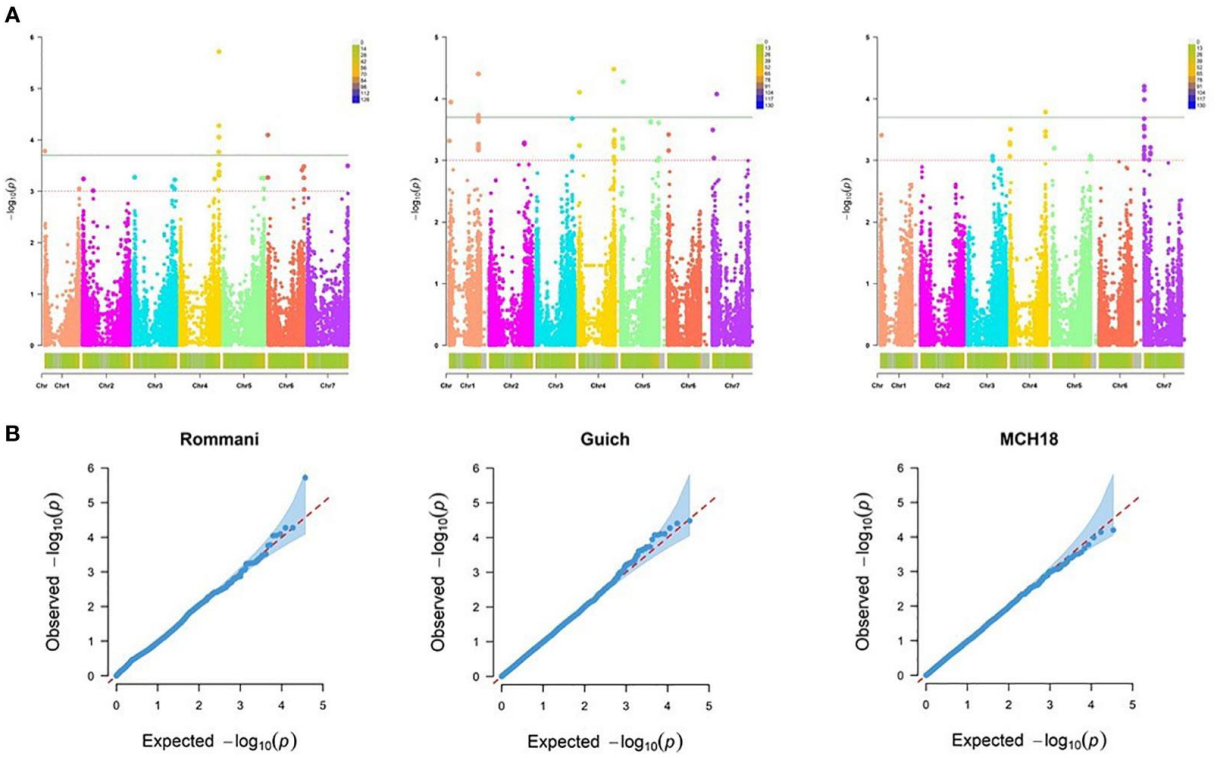


Figure III.12. Genome-wide association mapping of barley scald resistance at the seedling and adult plant stages. The Manhattan plots shows –log10 of p-values from genome-wide association mapping against the positions of SNPs on all chromosomes of barley at the adult plant stage at Marchouch (APR-MCH), Guich (APR-Guich18), and Rommani (APR-Rommani18(A). Quantile-Quantile (Q-Q) plots of marker-trait association at the adult plant stage using MLM model

Table III.2: A list of common SNP markers and their encoded candidate genes for the seedling and adult plant stage resistance to *Rhynchosporium commune* in barley AM2017 panel.

QTL	Marker	[a]Chr	Environments ([c]Allele effect)	[b]Position Morex V2 (Mbs)	Candidate gene	References
qSc.SRT7, qSc.APR13	JHI-Hv50k-2016-180771	3	SC-112 (-1.6) MCH18 (-11.34)	408.1375	CTP synthase family protein	Looseley et al. (2014)
qSc.SRT21, qSc.APR2	JHI-Hv50k-2016-438980	7	SC-112(0.691) MCH18 (7.7)	3.641	NBS-LRR disease resistance-like protein	Bjornstad et al. (2004), Thauvin et al. (2022)
qSc.SRT3, qSc.SRT12, qSc.APR2	JHI-Hv50k-2016-440870	7	SC-511(-0.6) SC-1122 (0.911) MCH18 (-9.3)	4.833	LRR receptor-like serine/threonine-protein kinase EFR, Disease resistance protein (CC-NBS-LRR class) family	Bjornstad et al. (2004), Thauvin et al. (2022)
qSc.SRT3, qSc.SRT12	SCRI_RS_226361	7	SC-511 (-0.637) SC1122 (-0.692)	4.8364	LRR receptor-like serine/threonine-protein kinase EFR, Disease resistance protein (CC-NBS-LRR class) family	Bjornstad et al. (2004), Thauvin et al. (2022)

[a] Chromosome
[b] Positions on the barley pseudomolecules Morex v. 2.0 2019
[c] Allele effect contributed by the respective marker on a 0–5 scale at the seedling stage. The negative allele effect decreases the diseases severity (resistance) and the positive allele effect increases the diseases severity (susceptibility).
The QTL indicated in bold text were significant with Bonferroni correction

III.3.6 Candidate genes and QTL alignment

Based on the annotation of the sequences in the barley reference genome, the majority of the MTAs were be located in genomic regions that are enriched with functional proteins associated with plant disease resistance and defense mechanisms. The BLAST searches revealed their homology with functional proteins/enzymes implicated in host plant disease resistance such as Serine/threonine-protein kinase Receptor-like protein kinase family protein, Disease resistance protein (CC-NBS-LRR class) family, receptor like kinases, Glutathion S-transferase, Nodulin MtN21 /EamA-like transporter family protein, Subtilisin-like protease, ABC transporter ATP-binding protein, wall associated kinase, Glycosyltransferase family 92 protein, Hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferase, ankyrin repeat

family protein, Pentatricopeptide repeat-containing protein, Sugar transporter protein, and WD repeat domain phosphoinositide-interacting protein. Among 15 SRT QTL, BLAST searches using sequences of peak SNP markers revealed the homology of 4 SNP markers with RLKs (receptor like kinases) and 2 SNP markers with NLR (Nucleotide-binding leucine repeat). Likewise, in case of APR, SNP markers associated with 7 QTL encode RLK (Annex III.4 and Annex III.5). Furthermore, 12 out of 15 SRT QTL, and 6 out of 12 APR QTL were validated by our study. However, 9 QTL on chromosomes 1, 2, 3, 4, 5, 6 and 7H reported in this study could be considered as novel.

III.4 Discussion

Rhynchosporium commune is one of the most destructive diseases of barley worldwide including in parts of North Africa and West Asia, requiring a continuous search for new sources of resistance and the development of associated molecular markers to introgress resistance loci to develop resistant varieties. In this study, we are reporting sources of resistance to *Rhyncosporium commune* from a collection of diverse barley genotypes at the seedling and at the adult plant stages. To our knowledge, this is the first GWAS study of scald resistance in spring barley from North Africa.

The successful inoculations at greenhouse and at the fields have allowed for efficient screening of AM2017 to scald at seedling and adult plant stages. A large number of sources of resistance were identified with differences in infection responses observed among the three *Rhynchosporium commune* isolates at the seedling stage and the at the three locations at the adult plant stage. Among the isolates, SC-1122 at the seedling stage, and the adult plant stage testing location APR MCH18 appeared to be the most virulent suggesting their use for further screening of germplasm. The high aggressiveness of SC-1122 could be due to the presence of functional NIP1 gene which affects virulence as reported by several authors (Mohd-Assaad et al., 2019; Stefansson et al., 2014).

The results confirmed the usefulness of AM17 constructed by breeders in supplying sources of resistance to scald and to leaf rust (Amouzoune et al., 2022). In general, breeders prefer to use as parents, either elite lines or released varieties from different countries included in the constructed panels. However, core collections and other subsets of landraces conserved in genebanks and accessions of wild *Hordeum* are also bringing sources of resistance (Hiddar et al., 2021). The monitoring of virulence of the pathogen and the screening of germplasm against the most virulent isolates and field populations could allow for a strategic deployment of effective resistance genes as an important component of integrated management of the disease.

Major genes can be either used sequentially or pyramided. However, the screening of the same material at both seedling and adult plant stages could allow the identification of both minor and major genes of resistance which can be deployed for a more durable resistance protecting barley at all stages. However, compilation of these genes requires the identification of QTLs associated with resistance.

Our results showed differences in infection response within the genotypes of AM2017 at the seedling stage to the three scald isolates. These differences could be attributed to diverse virulence spectrum of each *Rc* isolate. This notion was reinforced by conducting a race analysis of the three *Rc* isolates using barley differentials with known resistance genes. Based on the race analysis, it can be postulated that the *Rc* isolate SC-511 is NIP1 deficient as it showed compatible reactions both on Brier (*Rrs1*) and on Atlas (*Rrs2*).

Successful barley improvement depends on availability of genetic variation for breeders sought traits. Hence, with low genetic diversity in a given gene pool, crops become more vulnerable to biotic and abiotic stress (Sharma, 2017). The availability of the whole genome sequences and genotyping platforms have accelerated the identification, and the use of existing allelic diversity in barley germplasm. Compared with bi-parental mapping which is limited by allelic diversity among parents, GWAS can explore the existing allelic diversity and historical recombination events, resulting in reduced LD and superior mapping resolution. The LD of AM2017 panel was estimated to be 0.6 Mb (~0.4 cM), compared to earlier studies where LD of 3 to 5 cM was reported using different set of barley germplasm (Amezrou et al., 2018; Visioni et al., 2018; Visioni et al., 2020). In addition, AM2017 has an average SNP density of 37.09 SNPs/cM, which is much higher than earlier GWAS studies with 5 SNP/cM (Amezrou et al., 2018), 1 DArT marker per 1.5 cM (Comadran et al., 2009), and 1 SNP marker per 0.72 cM (Cockram et al., 2008; Pasam et al., 2012). Higher genetic diversity found in AM2017 is due to diverse origin of barley genotypes; 134 advance breeding lines from ICARDA's international spring barley breeding program, 161 registered cultivars from Asia, Africa, Europe, America, and 21 landraces. In addition, very high heritability estimates were observed for scald resistance at the adult plant stage (0.89-0.99) compared with heritability estimates at the seedling stage (0.40 to 0.58). Similarly, Feriani et al in (2012) reported a higher heritability of 64% for all scald isolates. Likewise, Xi et al (2019) also reported high heritability of scald resistance ranging from 0.57 to 0.91 under field conditions.

Successful barley improvement reported QTL conferring resistance to *Rhynchosporium commune* at the seedling stage have been identified through linkage analysis of biparental

mapping populations (Zhang et al., 2020). Though bi-parental mapping has resulted in the identification of eleven major scald disease resistance genes, *Rrs14* on chromosome 1H; *Rrs*1, *Rrs3*, and *Rrs4* on 3H; *Rrs6* and *Rrs9* on 4H; *Rrs13* on 6H; *Rrs2*, and *Rrs12* on 7H, most of the mapping studies converged on loci proximal of *Rrs1* (3H) and *Rrs2* (7H) (X. Zhang et al., 2020). Interestingly, the necrosis inducing protein (NIP1) is the product of avirulence gene *AvrRrs1* and conditions disease resistance in barley genotypes carrying *Rrs1*(Rohe et al., 1995; Steiner-Lange et al., 2003b; Stukenbrock & McDonald., 2009). Based on the position of flanking markers on Morex genome V2, *Rrs1* has been mapped on 445-455 Mbs on the chromosome 3H, and we also detected 15 SNPs in the same genomic region for *Rc* isolate SC-1122 as reported by numerous studies (Dyck & Schaller., 1961; Genger et al., 2003; Gønneød et al., 2002; Goodwin., 1990; Graner & Tekauz., 1996; Hofmann et al., 2013; Looseley et al., 2020; Patil et al., 2003; Wagner et al., 2008). Based on these findings, it can be postulated that only *Rc* isolate SC-1122 seems to have NIP1, whereas *Rc* isolates SC-611 and SC-511 did not harbor NIP1 as no QTL was detected on 3H. Likewise, *Rrs2* has been mapped on 5.2 Mb on 7H, and our genome scan has detected three QTL in the same genomic region; *qSc.SRT3* (4.83 Mb) for *Rc* isolate SC-511, *qSc.SRT12* (1.97-6.02 Mb) for SC-1122, and *qSc.SRT14* (7.66 Mb) for *Rc* SC-611. The same genomic region has been reported previously on 5.3 Mb by Daba et al. (2020) on 6.42 Mb, and in many other studies (Bjørnstad et al., 2004; Cheong et al., 2006; Gønneød et al., 2002; Wallwork et al., 2014; Yan et al., 2009). These results demonstrated that many new genomic regions were mapped at the vicinity of the previously mapped loci on chromosome 3H and 7H.

On chromosome 1H, the major resistance gene *Rrs14* introgressed from *Hordeum spontaneum* was mapped on 2.8 Mb (Garvin et al., 2000). We detected one APR QTL *qSc.APR9* (4.89-5.0 Mb) on the short arm of 1H which is in close proximity of *Rrs14* locus. Another APR QTL *qSc.APR3* on chromosome 1H at 484.55-485.81 Mb seems to be novel as it is located ~482 Mb away from *Rrs14* locus. Interestingly, the peak SNP JHI-Hv50k-2016-4445 (5.04 Mb) associated with the APR QTL *qSc.APR9* reduced the disease severity by 2.25 units (25%) and validated a QTL reported on 4.28 Mb by Wang et al in (2014).

On chromosome 2H, for *Rc* isolate SC-511 two SRT QTL *qSc.SRT1* (27.65 Mb), *qScSRT2* (579.22-583.15 Mb), and for *Rc* SC-611 one SRT QTL *qSc.SRT13* (17.76-19.21 Mb) were detected. Our study has validated a QTL reported by Daba et al., (2019) on 17.12 Mb and the same QTL was reported by Hofmann et al in (2013) and Von Korff et al (2005). Likewise, one APR QTL *qSc.APR21* has been reported by Looseley et al. (2012, 2014). s

On chromosome 3H, 49 SRT MTAs (48.03%) were grouped into 6 SRT QTL, spanning a physical distance from 394 to 454.93 Mb. Of the 6 SRT QTL from this study, five SRT QTL; *qSc.SRT7, qSc.SRT8, qScSRT9, qScSRT10,* and *qScSRT11* have been reported previously in different studies (Cheong et al., 2006; Coulter et al., 2019; Dyck & Schaller., 1961; Genger et al., 2003; Gønneød et al., 2002; Goodwin., 1990; Graner & Tekauz., 1996; Hofmann et al., 2013; Looseley et al., 2020, 2014, 2018b; Patil et al., 2003; Wagner et al., 2008). Whereas the SRT QTL *qSc.SRT6* (398.56-400.14 Mb) was novel. Furthermore, 4 APR MTAs were classified into 4 QTL and none of them overlapped with the mapped interval of *Rrs1*, which may indicate that the other genomic regions on 3H are important in conditioning adult plant stage resistance to scald. Alternatively, the field populations of *Rc* are either *AvrRrs1* deficient or have a mutated version of *AvrRrs1*. This can further be supported from our seedling screening assay where one of three *Rc* isolates SC-1122 detected QTL in *Rrs1* mapped locus. Three APR QTL; *qSc.APR13* (408-414 Mb), *qSc.APR4* (578.7-578.8 Mb), and *qSc.APR23* (597.95 Mb) have been reported previously (Cheong et al., 2006; Gønneød et al., 2002; Looseley et al., 2015).

On chromosome 4H, the major resistance gene *Rrs16* introgressed from *H. bulbosum* was mapped on 1.7 Mb (R. Pickering et al., 2006). Likewise, we detected two MTAs linked to *qSc.APR14* spanning a genomic region of 0.39-2.37 Mb with the peak SNP marker JHI-Hv50k-2016-225852 on 1.36 Mb, reducing the disease severity by 7.88 AUDPC units (Supplementary Tables S2 and S3). *Rc* QTLs have been reported in the same genomic region (Pickering et al., 2006; Wallwork et al., 2014; Wang et al., 2014). Similarly, *qSC.APR1* (605.57-606.16 Mb), *qSc.APR6* (602.22 Mb), and *qSc.APR11* (606.16-608.35 Mb) have been reported by several authors (Jensen et al., 2002; Looseley et al., 2012; Zantinge et al., 2019). Five APR QTL were detected on 5H conditioning resistance to scald at two field locations. The two QTL *qSc.APR17* (Guich18) and *qSc.APR24* (AR18) are located between 550.77 to 553.49 Mb may represent the same QTL, and Looseley et al (2012) also reported the same genomic region to be associated with scald resistance. Likewise, Daba et al. (2019) mapped a QTL on 587.44 Mb which coincides with two APR QTL; *qSc.APR18* (586.95 Mb) and *qSc.APR25* (582-589 Mb) reducing the disease severity by 0.9 (10%) to 1.13 (14%) units. Of the 5 APR QTL, only 1 QTL (*qSc.APR7*; 37.99 Mb) was novel. Interestingly, the peak SNP JHI-Hv50k-2016-343816 (553.49 Mb) associated with *qSc.APR24* reduced the disease severity by 1.78 units (20%) and can be used as a functional marker in Marker Assisted Selection (MAS).

In previous studies, two major scald resistance genes, *Rrs13* (11.8 Mb) and *Rrs18* (25.6 Mb) were mapped on the chromosome 6H (Abbott et al., 1995; Coulter et al., 2019; Genger et al., 2003; Read & Oram., 1995). Among the three APR QTL identified on 6H, *qSc.APR19* (23.712-23.746 Mb) was detected in close proximity of *Rrs13* and Shtaya et al in (2006) also mapped a QTL on 21.3 Mb. Further, another APR QTL *qSc.APR26* (4.34 Mb) was detected by Jensen et al (2002) from the cultivar Alexis, and by (Cheong et al., 2006) from the cultivar Keel in two different studies. No SRT QTL was detected on 6H for the three *Rc* isolates tested. Of the 102 MTAs at SRT, 48 (47%) were mapped on the chromosome 7H and constituted 6 QTL of which 5 QTL were validated by our study and 1 QTL was novel. The SRT QTL *qSc.SRT5* (614.71-615.55 Mb) may represent the same QTL reported by Wang et al (2014) and Thauvin et al (2022) on 7H.

The markers/QTL stable across environments would be ideal candidates for MAS (marker assisted selection) and genomic selection (GS). Two common SRT QTL were detected. We detected two SNPs JHI-Hv50k-2016-440870 (4.832 Mb) and SCRI_RS_226361 (4.863 Mb) associated with *qSc.SRT3* and *qSc.SRT12* against *Rc* SC-511 and SC-1122 on 7H, respectively and may represent the same QTL. About 9 significant SNPs were encompassing a genomic region of 1.97-10.86 Mb on 7H, conditioning resistance to all the three *Rc* isolates. These may represent different alleles of *Rrs2* or different resistance genes. In addition, seven common APR QTL were detected across environments. Seven SNPs encompassed a genomic region of 602.22-608.35 Mb on 4H representing *qSc.APR1* (APR-MCH18), *qSc.APR16* (Guich18), and *qSc.APR11* (AR18) which may represent the same QTL. Likewise, the close proximity of *qSc.APR17* (550.77 Mb; Guich18) with *qSc.APR24* (553.49 Mb; AR18), and *qSc.APR18* (586.95 Mb; Guich18) with *qSc.APR25* (582.53-589.44; AR18) on 5H most probably represent the same QTL. A list of 4 common SNPs were shared between SRT and APR. Similarly, *qSc.APR20* (10.12 Mb; Guich18) and *qSc.APR27* (10.74 Mb; AR18) represent the same QTL. These SNPs can be used for MAS and genomic selection platforms to accelerate gene pyramiding efforts.

The understanding of host-pathogen interaction at genetic level is quite important for identifying and deploying scald resistance. Plants have a multilayered inducible defense system. Their first layer of defense is based on extracellular pattern recognition receptors (PRRs) which recognize conserved microbe/pathogen/damage-associated-molecular patterns (MAMPs/PAMPs/DAMPs). Mostly PRRs are membrane anchored receptors with an ecto-domain for ligand binding for example leucine-rich-repeat (LRR) domain or a chitin binding

(LysM) domain (Miya et al., 2007) with or without an endo- kinase domain like in receptor like kinases (RLKs) or receptor-like proteins (RLPs) which require a co-receptor for cellular signaling. The activation of PRRs induces transcriptome reprogramming and biosynthesis of antimicrobial compounds to limit pathogen's ingress. However, successful pathogens use avirulence gene products (effectors) to subvert host defense responses. The second layer of defense is initiated upon recognition of pathogen secreted effectors by nucleotide-binding leucine-rich repeat (NLR) receptors either directly (gene-for-gene model) or indirectly by guarding and sensing conformational changes in the host virulence targets of the effectors which results in a localized cell death, a phenomenon referred here as effector triggered immunity (ETI) (Jones & Dangl., 2006). *R. commune* is an hemibiotrophic pathogen of barley and a gene-for-gene interaction has been proposed with its host. Three necrosis inducing proteins (NIP1, NIP2, and NIP3) have been described from *Rc*, where NIP1 is expressed in spores, but NIP2 and NIP3 are expressed upon host infection (Kirsten et al., 2012). NIP1 serves the function of an effector by inducing leaf necrosis, and as an elicitor to induce *Rrs1* dependent defense response (Rohe et al., 1995; Steiner-Lange et al., 2003a; Wevelsiep et al., 1993). That is why NIP1 has been under positive selection as indicated by the presence of two paralogous and copy number variations. From a global collection of 614 *Rc* isolates, Schürch et al in (2004) reported absence of NIP1 deletion in 45% of the isolates, whereas, NIP2 and NIP3 were present in all isolates. Different *Rc* isolates either lacking NIP1 or mutated NIP1 can overcome cultivars carrying *Rrs1* (Mohd-Assaad et al., 2019; Stukenbrock & McDonald., 2009). Therefore, the identification and deployment of both qualitative and quantitative resistance genes offers durable control of this rapidly evolving pathogen. None of the major resistance genes against scald have been cloned and functionally characterized, hence the molecular mechanism of disease resistance conditioned by *Rrs1* and *Rrs2* remains elusive. *Rrs1* does not encode a receptor for NIP1 but triggers resistance response upon recognition of NIP1 by an unknown co-receptor. With the availability of barley genome and predicted protein repertoire, functional homology-based BLAST searches using marker sequences associated with mapped QTL will help in candidate gene identification. Interestingly, the QTL *qSc.SRT11* was collocated within *Rrs1* mapped region on the chromosome 3H which was reported in different studies (Bjornstad et al., 2002; Dyck & Schaller., 1961; Gønneød et al., 2002; Goodwin., 1990; Graner & Tekauz., 1996; Hofmann et al., 2013; Looseley et al., 2020; Patil et al., 2003; Wagner et al., 2008).

RLKs contain an ectodomain, a transmembrane spanning region, and an intracellular cytoplasmic kinase domain to relay the pathogen recognition signal (Tör et al., 2004). The SNP

marker JHI-Hv50k-2016-124224 associated with *qSc.SRT15* on chromosome 2H (632.26 Mb) encodes a serine/threonine-protein kinase (HORVU.MOREX.r3.2HG0199700), reducing the disease severity by 1.35 units (27%). Likewise, the SNP marker JHI-Hv50k-2016-4445 associated with *qSc.APR9* on chromosome 1H (5.041 Mb) also encodes a serine/threonine-protein kinase (HORVU.MOREX.r3.1HG0002370), reducing the disease severity by 2.25 units (25%). The Serine/threonine protein kinases phosphorylate the OH group of two amino acids, serine or threonine of the target protein, and this protein phosphorylation process plays an important role in the disease resistance pathway. In wheat the *Pm21* locus conditions durable resistance to wheat powdery mildew (*B.graminis* f.sp. *tritici (Bgt)*) (He et al., 2022). A putative serine/threonine-protein kinase (*Stpk-V*) was cloned from the *Pm21* locus and its transient expression in a susceptible wheat variety Yangmati158 rendered broad spectrum durable resistance to powdery mildew. Whereas virus induced gene silencing of *Stpk-V* in resistant wheat cultivar rendered it highly susceptible to *Bgt* (Cao et al., 2011; Chen et al., 1995). In barley, the leaf rust resistance gene *Rph22* encodes a lectin receptor like kinase and some of the RLKs may possibly be involved in basal defense if an analogy is to be drawn with other extracellular PTT receptors (Wang et al., 2019). Likewise, in rice, an RLK (*Xa21*) confers resistance to bacterial blast caused by *Xanthomonas oryzae* pv. *Oryzae* (Song et al., 1995; Tör et al., 2004). We found 6 APR QTL (*qSc.APR6, qSc.APR7, qSc.APR18, qSc.APR19, qSc.APR21, and qSc.APR24*), and 5 SRT (*qSc.SRT2, qSc.SRT3, qSc.SRT11, qSc.SRT12, qSc.SRT15*), where several SNP markers encode RLK as candidate genes. The co-location of candidate genes within families with known role in disease resistance such as RLK, NLR and wall associated proteins can help identify new allelic variants of existing genes or completely new resistance genes.

Sugar transporters mediate sugar transport and are one of important players in plant-pathogen interaction (Breia et al., 2021). A SNP marker JHI-Hv50k-2016-268095 is associated with a APR QTL *qSc.APR11* on 4H at 606.1682 Mb encodes a sugar transporter (HORVU.MOREX.r3.4HG0411610). This SNP marker reduced the disease severity by ~20% in MCH18. Recently cloned what leaf rust resistance gene *Lr67* encodes a sugar transporter 13 (SAT13), which plays a crucial role in conditioning partial resistance to all wheat rusts and powdery mildew (Milne et al., 2019). Its susceptible variant (*Lr67sus*) is a functional sugar transporter and its expression is upregulated upon pathogen invasion, whereas its dominant variant (*LR67res*) is unable to transport sugar which is required by wheat rusts and powdery mildew. Likewise, *Xanthomonas oryzae* pv. *oryzae* secreted TAL (Transcription-activator like)

effector upregulate the expression of a sugar transporter *Os SWEETY11* in rice (Chen et al., 2010). Similar findings have been reported for Arabidopsis-*Botrytis cinerea*, grape vine interaction with powdery mildew (*Erysiphe nectar*) and downy mildew (*Plamopara viticola*) (Hayes et al., 2010; Lemonnier et al., 2014). Sugar transporters are quite conserved in plant kingdom and a similar role in disease resistance can be envisioned for these transports in barley-*Rhynchosporium commune* interactions. The nodulin-like proteins belong to a group of aquaporins and they have been implicated in the transport of various solutes in plants in addition to their interaction with various plant pathogens. In barley, the sequences of two SNPs on 7H, JHI-Hv50k-2016-483974 (*qSc.SRT4*, 393.195 Mb) encode putative Early Nodulin-like protein. Interestingly, Gyawali et al in (2018) also reported a QTL (*Rcs-qtl-7H-32.81*) for spot blotch resistance in barley on 7H where the SNP marker 11_20162 encoded an Early Nodulin-93 like protein. The ABC transporter proteins condition disease resistance by secreting the anti-fungal products into the apoplast like PEN3 in Arabidopsis against *B. cinerea* (Y. He et al., 2019), and *Lr34* against wheat leaf rust (Krattinger et al., 2009). We found that ten adjacent SNP markers (453.535-453.764 Mb), in the interval of *qSc.SRT11* on 3H, were flanked by ABC transporter B protein (HORVU.MOREX.r3.3HG0282510). In wheat, *Lr34* provided resistance against wheat stem rust, stipe rust, and powdery mildew (Rinaldo et al., 2017) in addition to powdery mildew and rust resistance in barley (Boni et al., 2018), and against rice blast (Krattinger et al., 2016). We speculate an important role of ABC transporter proteins in resistance against *R. commune*. Our study detected several SNP markers encoding functional proteins which have been involved in plant defense against diverse fungal pathogens. The newly detected QTLs can be applied in barley breeding program and the information could be employed by breeders for marker assisted selection, fine mapping, gene cloning for improving resistance to scald disease.

III. 5 Conclusion

The identification of diverse source of resistance and molecular tagging of new genomic regions associated with resistance to *R. commune* is crucial for future marker-assisted selection and genomic selection for continuous improvement of barley germplasm. Our GWAS was efficient in genetic dissection of scald resistance. Several SNPs were located within already known QTL associated with scald disease, in addition to the identification of novel seedling stage resistance QTL on the chromosomes 2H, 3H, and 7H, and the adult stage resistance QTL on the chromosomes 1H, 4H, 5H, 6H, and 7H. Durable scald resistance requires further understanding of *R. commune* pathogen population dynamics and efficient introgression of major and minor genes using molecular markers.

Chapter IV: Exploring accessions of *Hordeum spontaneum* for resistance to scald (*Rhynchosporium commune*) and identification of associated genomic regions

IV.1 Introduction

Hordeum vulgare subsp. *spontaneum* (K. Koch) Thell., commonly referred to as Spontaneum, is recognized as the ancestral form of cultivated barley. It holds significant value for barley breeding programs due to its genetic contributions, which offer resistance to diseases like powdery mildew, leaf scald, and leaf rust (Řepková et al., 2006). Additionally, it carries desirable attributes for enhancing yield (Von Korff et al., 2006), and provides resilience against environmental stresses such as drought and extreme temperatures (Chen et al., 2008; Lakew et al., 2013). *Spontaneum* is also prized for its contribution to end-use qualities, notably in improving the malting characteristics of barley (Erkkila et al., 1998; von Korff et al., 2008).

Barley (*Hordeum vulgare L.*) is one of the most salutary and the earliest domesticated cereals, grown in the "Fertile Crescent" 10,000 year ago (Badr et al., 2000; Harwood., 2019). Globally, barley ranked the fourth cereal in term of produced tons and harvested area (FAOSTAT. 2018). Russian Federation, Australia, Europe, United Kingdom, Canada, North America, and Asia are the major producer of barley (Harwood., 2019). It stands out as a crop where the utilization of Crop Wild Relatives (CWR) in breeding programs is especially notable (Maxted & Kell., 2009). The Fertile Crescent is widely recognized as the main origin and domestication site for barley, as indicated in several studies (Badr et al., 2000; Zohary et al., 2012). However, further research points to additional barley domestication occurrences in regions east of the Fertile Crescent (Morrell & Clegg., 2007) as well as in Tibet (Dai et al., 2012), Ethiopia, and the Western Mediterranean (Molina-Cano et al., 2005; Orabi et al., 2007).

Barley and has multiple uses such as animal feed, brewing and malt, also is largely used on the modern human diet in many developing as well as developed countries and possesses many health benefits (Blake et al., 2011). Particularly in Japan, 90 of barley is consumed as human food (Aoki et al., 2011). It is also consumed in Morocco, Ethiopia, India and China. In addition, barley has a potential to adapt in a wide range of different environmental conditions from desert, high altitudes to the high altitudes (Ceccarelli et al., 1992; Hecht et al., 2016; Serna-Saldivar., 2016; Srivastava & Damania., 1989). Around the globe barley can grow in the spring and winter seasons. However, its quality and productivity are affected by various biotic factors include viruses, fungi, and bacteria (Pessarakli., 2019). Among these phytopathogens, *Rhynchosporium commune* is one of the most serious and destructive foliar diseases, known as scald or leaf

blotch, which is spread around the world and found in all continents, but more is frequent and devastating under cool and semi-humid regions (Zhan et al., 2008a). Yield loss can amount to more than 40% (McDonald et al., 1999b; Paulitz and Steffenson., 2011; Xi et al., 2000). Infected seeds play a significant role in the rapid spread of the disease and the survival of the fungus (Salamati et al., 2000).

Control of scald disease is becoming hard and difficult, due to the high genetic diversity of the pathogen populations, which are able to change rapidly (Salamati et al., 2000).

Another challenge facing the control of scald disease is its ability to survive, colonize and sporulate on the resistant varieties, which can be a source of inoculum for further infections (Atkins et al., 2010). PCR are able to detect symptomless presence of *Rhynchosporium* on the seeds (Lee et al., 2002), which allow the transmission of the pathogen from the seed to seedling (Fountaine., 2005). Using fungicides and cultural practices has not proved to be the most effective and sustainable strategies for the management and controlling scald disease and resistance of *R. commune* populations to multiple fungicides has been reported (Carisse et al., 2000; Shipton et al., 1974).

Genetic control is the most economical and environmentally-friendly way to control scald disease, but become ineffective after large adoption of resistant varieties (Oxley et al., 2003), calling for a continuous supply of sources of resistance to cope with new virulence.

Genetic resources are key for crop improvement as they offer valuable sources of genes for use in breeding new varieties with higher yields and adequate levels of resistance to major diseases. Wild species of cultivated crops, are an important reservoir of genes for future agriculture, and the wild relative species in primary, secondary and the tertiary gene pools, may have a wider spectrum of resistant genes than the cultivated species (Harlan & de Wet., 1971).

Wild Barley (*Hordeum vulgare* subsp. *spontaneum)* has a diploid genome (2*n* = 14), with the availability of high-quality reference genome sequence (Harwood., 2019). Several studies have been suggested that *Hordeum spontaneum* is an important and very rich source of genes for biotic and abiotic stresses (Çelik et al., 2017; Karakaya et al., 2016). Genes conferring resistance from *H. spontaneum* have been successfully transferred into cultivated barley, providing enhanced protection against various diseases and pests. This includes defenses against leaf rust (Steffenson et al., 2007), powdery mildew (Jørgensen., 1992; Moseman, Nevo, & Zohary., 1983), scald (Abbott et al., 1991), and the aphid *Rhopalosiphum padi* (Åhman & Bengtsson, 2019).

Resistance genes controlling scald disease have been reported from wild barley (Garvin et al., 2000; Genger et al., 2003; Von Korff et al., 2006; Yun et al., 2005). As a result of their superior

characteristics and for future research purposes, preservation of wild barley in situ and ex situ conditions would be necessary (Nevo., 2013).s

Exploitation of genetic diversity found in wild relatives, landraces, and other underutilized genetic resources of barley through efforts of pre-breeding is a fundamental step in the breeding pipeline, bridging the gap between the conservation of genetic resources and the development of new varieties that meet the evolving needs of farmers, industry, and consumers.

The present study aims to identify sources of resistance to scald in a collection of *Hordeum spontaneum* from ICARDA's gene-bank, to assess its genetic diversity and the population structure, and to identify quantitative trait loci associated with resistance to scald.

IV. 2 Materials and Methods

IV.2.1 Plant material

A total of 103 accessions of *Hordeum spontaneum* collected from various parts of the world and conserved at ICARDA's gene bank were evaluated for scald resistance (Figure IV.1).

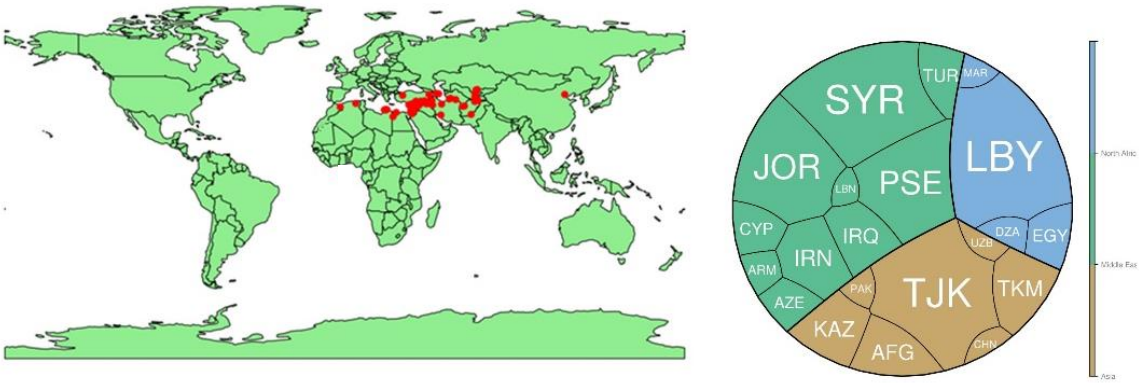


Figure IV.1. Geographic distribution of 103 accessions of *Hordeum spontaneum* accessions

IV.2.2. *Rhynchosporium* Isolation

Scald-infected barley leaves were collected from naturally affected barley fields across various agro-ecological zones in Morocco during disease surveys conducted from 2015 to 2018. To isolate *R. commune*, we followed a series of steps. Initially, we immersed the scald-infected barley leaves in sterile distilled water for 15 minutes, subsequently, the leaves underwent surface sterilization through a 10% sodium hypochlorite solution for 30 seconds, followed by a 50% ethanol treatment for 15 seconds. Then, we rinsed the leaves thoroughly with sterile distilled water three times and finally, the treated leaves were dried between two layers of sterile Whatman filter paper. The leaf segments were placed in darkness at a temperature of 14°C for

two weeks on Lima Bean agar (LMA) that was supplemented with Kanamycin and Streptomycin (50 mg per liter). After this incubation period, sporulating white or pinkish colonies were streaked onto new LBA plates for the purpose of preparing single conidial isolates. About 50 isolates of *R. commune* were isolated and conserved in the pathology ICARDA labroratory. The virulence spectrum of 15 isolates was studied on a set of differentials namely Atlas, Atlas 46, Brier, CI3515, CI4364, Jet, La Masita, Osiris, and Turk, and allowed the choice of the most virulent SC-1122 and SC-511 used in the screening at seedling stage.

IV.2.3. Assessment of the disease at seedling plant stage

Assessment of plant infection is based on morphology, lesion size, chlorosis and necrosis. Each accession was represented by approximately four seeds, which were sown in individual 3.8 cm diameter and 14 cm deep cones filled with peat moss and supplemented with 14-14-14 NPK fertilizer in three replications using two susceptible (Tissa and Tocada) and two resistant (Atlas 46 and ICARDA 4) checks The seedlings of the *Hordeum spontaneum* accessions were grown under controlled conditions within a growth chamber (Model MC1750; Snijder Scientific, Tilberg, Netherlands) at ICARDA, Rabat, Morocco. The growth chamber was maintained at a photoperiod of 16 hours of light and 8 hours of darkness at a constant temperature of 20 ± 1°C. For assessing seedling resistance, two virulent isolates of *R. commune*, namely SC-511 and SC-1122 collected from different locations (Marchouch, Sidi Allal Tazi) were used. The inoculum was prepared by gently rubbing the agar surface of 10-12 days old LBA plates with a sterile glass slide, followed by filtration through a double layer of cheesecloth.

Inoculation was carried out on approximately two-week-old seedlings, with the second leaf fully extended. A spore suspension containing 5×10^5 conidia/ml, supplemented with 0.01% Tween 20 as a surfactant, was used for inoculation. Subsequently, the seedlings were incubated in a dark environment at 100% relative humidity for 72 hours at 15°C within the growth chamber.

Following the incubation period, the seedlings were relocated to a greenhouse with a temperature regime of 20°C during the day and 16°C during the night and a photoperiod of 16 hours of light and 8 hours of darkness. Infection types were recorded at 16 days post-infection (dpi) using a disease rating scale ranging from 0 to 5, as per the scale introduced by Salamati and Tronsmo (1997), where 0 = I (Immune), 1 = R (Resistant), 2 = MR (Moderately Resistant), 3 = MS (Moderately Susceptible), 4 = S (Susceptible), and 5 = HS (Highly Susceptible).

IV.2.4. Assessment of the disease at adult plant stage

The field experiments were conducted in Morocco during the 2018-2019 cropping season at various locations, including the INRA experimental stations at Marchouch (MCH; coordinates: 33° 56' N, 6° 63' W) and at Guich (coordinates: 33° 58' N, 6° 51' W).

In these trials, each test entry was sown in paired rows, with each row measuring 1 meter in length and spaced 0.5 meters apart. The statistical augmented desing was used, where the test entries were not replicated while two susceptible (Tissa and Tocada) and two resistant checks (Atlas 46 and ICARDA 4) are replicated in each block.

To create conditions of elevated disease pressure, a protective border was established around each experimental block. This border consisted of a mixture of barley genotypes known to be susceptible to scald disease, including Tissa, Aglou, Tiddas, Fleet, Adrar, Shepherd, Baudin, and Alester. This arrangement was employed to facilitate a rigorous assessment of disease resistance in the test entries.

Beginning at Zadoks scale GS30 (Zadoks and Board, 1974) and following a 10-12 days interval, the field trials were intentionally infected four times. This infection process was carried out using a knapsack sprayer and an inoculum mixture comprised of 15 different isolates of *R. commune*. These isolates were gathered from various agro-ecological regions within Morocco. To ensure the establishment of the disease, barley residues infected with scald from the previous growing season were evenly distributed across the trial area. Additionally, the development and spread of the disease were encouraged by providing regular mist irrigation in the late afternoons. At growth stages GS 73-75, disease severity was assessed using a 0-9 scale following the guidelines of Saari & Prescott (1975). The evaluated accessions were categorized into five classes: those scoring from 0 to 2 were deemed resistant, those with scores of 3 to 4 were categorized as moderately resistant, genotypes with scores between 5 to 6 were considered moderately susceptible, those with scores of 7 to 8 were labeled as susceptible, and any entries scoring 9 were classified as highly susceptible.

The area under the disease progression curve (AUDPC) was calculated using the equation provided by Jeger and Viljanen-Rollinson in (2001).

IV.2.5. Phenotypic data analysis

To assess the frequencies of various reaction classes to *R. commune* at both the seedling and adult plant stages, we employed R software (R Core Team., 2019). Additionally, Venn diagrams were created to visually depict the common occurrences of resistant entries shared among the two isolates and among the two fields.

A t-test was used to determine if there is a statistically significant difference between the isolates (SC-511 and SC-1122) at the seedling plant stage, and between the pathogen population under field conditions. We compared the calculated p-value to a predefined significance level (alpha), alpha= 0.05.

IV.2.6. Genotyping, population structure, and linkage disequilibrium of *Hordeum spontaneum*

Genomic DNA isolation was carried out during the GS12 growth stage, utilizing lyophilized young leaf tissue obtained from a single plant at the Cereal Crop Research Unit, USDA-ARS, Fargo, North Dakota, USA, following the method outlined by Slotta et al. (2008).

For SNP genotyping, the Illumina iSelect 50K SNP array designed for barley (Illumina, San Diego, CA, USA) was employed, with adherence to the manufacturer's instructions as described by Bayer et al in (2017). Subsequently, 26,253 SNP markers underwent genetic analysis after quality control measures were applied, including the elimination of monomorphic markers, markers with missing values exceeding 20%, and those with a minor allele frequency (MAF) lower than 5%. To assess the population structure of *Hordeum spontaneum*, we utilized the filtered SNP markers to estimate individual admixture coefficients. This analysis was conducted through the implementation of the sparse Non-negative Matrix Factorization (sNMF) algorithm, which is part of the R package LEA (Frichot & François., 2015).

The assessment included the use of the cross-entropy criterion from the sNMF function to evaluate various potential populations (K) ranging from K=2 to K=20, following the approach described by (Alexander and Lange., 2011; Frichot et al., 2014). For each K value, we configured the number of iterations to 200, the number of runs to 10, set alpha to 10, and established a tolerance level of $10^{\wedge 5}$. Accessions were classified as admixed or assigned to subgroups based on an 80% membership criterion. Additionally, we performed a Principal Component Analysis (PCA) using the 26,253 filtered SNP markers, employing TASSEL version 5.0. to further explore the relationships among the 103 genotypes, we computed the kinship matrix (K) using the filtered set of SNP markers in TASSEL 5.0, as described by Bradbury et al. (2007).

We assessed linkage disequilibrium (LD) for all pairs of loci using SNP markers with known positions. To visualize the genome-wide LD decay, we created plots by graphing intra-chromosomal r^2 values against physical distances in Mb, following the methodology outlined by Purcell et al. (2007).

IV.2.7. Genome-wide association study

The association mapping was conducted on 103 wild barley genotypes to identify genomic regions associated with scald disease resistance at both plant growth stages. GWAS analysis was performed by integrating phenotypic and genotypic data (Bradbury et al., 2007; Lipka et al., 2012). Two general linear models (GLM+Q), and (GLM+ PCA), and two mixed linear models, (MLM+Q+K), and (MLM+PCA+K), were tested by taking into account kinship (K matrix), population structure (Q matrix), and principal coordinate analysis (PCA) as covariates to control false positives, and were implemented in GAPIT3. By using these models, the study aimed to reduce the likelihood of false positives, which are non-causal associations that appear significant due to population structure, kinship, or other confounders rather than a true genetic relationship with the trait of interest. A GWAS threshold P-value of [−log10(P-value) ≥ 3.0 or $P < 0.001$. was used for declaring significant-marker trait associations. The genetic linkage maps were drawn using barleymap (Cantalapiedra et al., 2015).

IV.2.8. Candidate genes and alignment analysis

To identify potential candidate genes involved in scald resistance at both plant growth stages, significant markers were aligned against the physical map in the barleymap (Cantalapiedra et al., 2015). The search for candidate genes was specifically oriented towards identifying proteins that possess functional domains known to be involved in the mechanisms of plant disease resistance. The sequences of markers linked to previously identified Quantitative Trait Loci (QTL) were sourced from the Grain Genes database (https://wheat.pw.usda.gov/blast/). Subsequent to retrieval, these marker sequences underwent positional verification against the barley reference genomes, namely Morex version 2.0, which was released in 2019, and the updated Morex version 3 pseudomolecules made available in 2021. This verification process was conducted through the Barleymap pipeline, a tool and methodology developed and described by Cantalapiedra et al in (2015). The identified potential candidate genes were further submitted to the 'The BarleyExpDB ' (expression Visualization and Integration Platform) (http://barleyexp.com/) for *in-silico* gene expression analysis ((T. Li et al., 2023).

IV.3 Results

IV.3.1. Reaction of *Hordeum spontaneum* to scald disease at the seedling stage

It was very easy to score the infection responses of the accessions induced by *R. commune* isolates. In the susceptible and highly susceptible accessions, chlorosis lesions fully covered the leaf area of infected plants. Scald infections exhibited a high level of success in both

controlled and field conditions, as evidenced by the consistent infection of susceptible accessions and checks. At the adult plant stage, susceptible checks, Tocada and Tissa, recorded an average disease severity score of 8, whereas the resistant checks, ICARDA4 and Atlas 46, demonstrated a notably lower average score of 1. Similarly, during the seedling stage, when exposed to the SC-511 isolate, resistant check varieties displayed minimal infection with an average score of 0, while the susceptible checks exhibited an average score of 5. In the case of the SC-1122 isolate, the average scores for the resistant and susceptible checks were 1 and 4, respectively.

A high variation response was found in *Hordeum spontaneum* accessions to the two *R. commune* isolates, ranging from Immune to highly susceptible reactions.

The Rc isolate SC-511showed the highest percentage of the immune (18%) genotypes, while The Rc isolate SC-1122 did not trigger any immune reactions. SC-1122 isolate demonstrated a higher percentage of resistant (37%) and moderately resistant (22%) reactions, compared to SC-511with (27%) and (8%) respectively. Furthermore, SC-1122 also induced a lower percentage of susceptible (4%) reactions and did not elicit any highly susceptible reactions. However, both isolates had a similar percentage (20%) of accessions with moderately susceptible reaction (Figure IV.2.A). Ten accessions were resistant and only two accessions were moderately resistant to the two isolates (Figure IV.2.B).

IV.3.2. Reaction of *Hordeum spontaneum* to scald disease at the adult stage

At the adult plant stage, the percentage of accessions for scald disease reactions differed based on the location and the pathogen population. MCH had a slightly higher percentage of resistant (29%) and moderately (31%) reactions compared to Guich. However, both locations had similar percentages of moderately susceptible class (25%) at MCH and (22%) at Guich. Whereas the highest percentage (12%) of the susceptible accessions were detected at MCH (Figure IV.2.C). Only five accessions were resistant at the adult plant stage under field conditions, and about seven were moderately resistant (Figure IV.2.D).

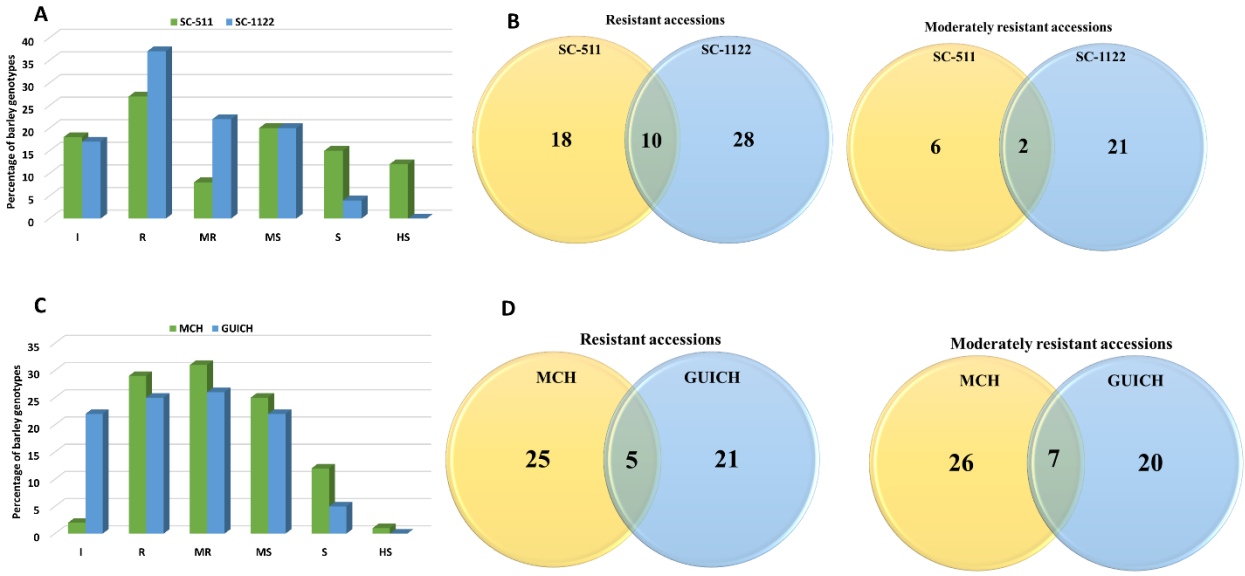


Figure IV.2. Frequency distribution of leaf scald resistance in 103 barley genotypes of Hordeum spontaneum at the seedling stage against R. commune isolates, SC-511, and SC-1122 (A). Venn diagrams showing barley genotypes with combined resistance at the seedling stage to the two *R. commune* isolates under controlled conditions (B). Frequency distribution of leaf scald resistance in 103 barley genotypes of *Hordeum spontaneum* at the adult plant stage at Marchouch (MCH), GUICH (GUICH) (C). Venn diagrams showing barley genotypes with combined resistance at the adult plant stage at two field locations (D).

The T-tests showed highly significant reactions *H. spontaneum* accessions against the two isolates at the seedling stages (P-value = 0.0085) and between locations at adult plant stage ((p-value = $3.84902E^{-05}$). (Table IV.1).

Table IV.1: The p-value of the T-tests comparing the reactions of the set of *Hordeum spontaneum* to the two isolates of *Rynchosporium. commune* (SC-511 and SC-1122) at the seedling plant stage and between the pathogen populations at adult plant stage at Marchouch and Guich locations

	Seedling plant stage	**Adult plant stage**
	SC-511 and SC-1122	MCH and GUICH
P-value	0.008514325	3.84902E-05

IV.3.3. SNP Marker Distribution and Density

The results revealed that each chromosome exhibits variation in terms of SNP number, density, allele frequency, polymorphism information content, and heterozygosity (Table IV.2). These metrics collectively provide insights into the genetic diversity and variation within the studied population. After eliminating monomorphic markers, and those with a minor allele frequency (MAF) lower than 5%, a total of 26,253 polymorphic SNP markers were retained and employed

for subsequent genetic analysis. The distribution of SNP markers across different chromosomes in wild barley showed that chromosome 5H has the highest number of SNP markers (4820), followed by the chromosomes 2H and 3H with 4398 and 4083 SNP markers, respectively. While the lowest number of SNPs amongst the seven chromosomes are found on chromosome 1H (2928).

The Mean MAF appears relatively consistent across all chromosomes, with values ranging from 0.2 for Chromosome 1H to 0.22 for chromosomes 3H and 7H. The PIC values which provide a measure of the informativeness of the SNP marker ranged from 0.29 to 0.31 among the seven wild barley chromosomes. Chromosomes 2H, 3H, 5H, and 6H consistently showed high Mean PIC values (0.3), indicating that the markers on these chromosomes are particularly informative. Chromosome 7H stands out with a slightly higher Mean PIC (0.31), suggesting potentially unique genetic characteristics. Mean Heterozygosity values were close to 0 indicating that the population is relatively homogenous showed and with low rates of heterozygosity.

The SNP density indicating the variations in the distribution of markers across chromosomes, differed among chromosomes with 5H having the highest density (6.39 SNP/Mb), followed by crhomosomes 2H, 3H, and 7H, while chromosomes 1H, 4H, and 6H have intermediate densities ranging from 5.73 to 5.86 SNP/Mb (Table IV.2). Furthermore, the distribution and the SNP density were higher in proximal and distal portions compared to pericentromeric regions of the chromosomes (Figure IV.3).

Table IV.2: Representation of genome-wide distribution for a dataset of 40,342 SNP markers across the seven chromosomes of *hordeum spontaneum*

Chr	No. SNP	Start Pos	End Pos	Length (Mb)	Density (SNP/Mb)	Mean MAF	Mean PIC	Mean Heterozygous
1H	2928	44868	750535067	750.49	3.90	0.20	0.29	0.019
2H	4398	401	767958191	767.96	5.73	0.21	0.30	0.019
3H	4083	134313	697989230	697.85	5.85	0.22	0.30	0.016
4H	3003	50710	738362577	738.31	4.07	0.21	0.29	0.019
5H	4820	254339	755094798	754.84	6.39	0.21	0.30	0.019
6H	3170	329535	732688792	732.36	4.33	0.21	0.30	0.016
7H	3851	174327	656988497	656.81	5.86	0.22	0.31	0.021
Total	**26253**	**-**	**-**	**-**	**-**	**0.21**	**0.30**	**0.018**

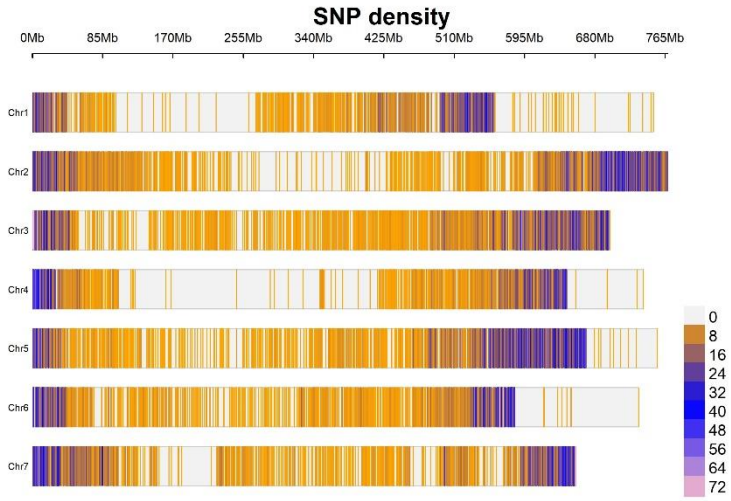


Figure IV.3. SNP density across the Hordeum spontaneum genome representing number of SNPs within 1 Mb window size. The horizontal axis represents the length of the chromosomes in megabases (Mb), with varying colors indicating the density of SNPs.

IV.3.4 Linkage disequilibrium analysis

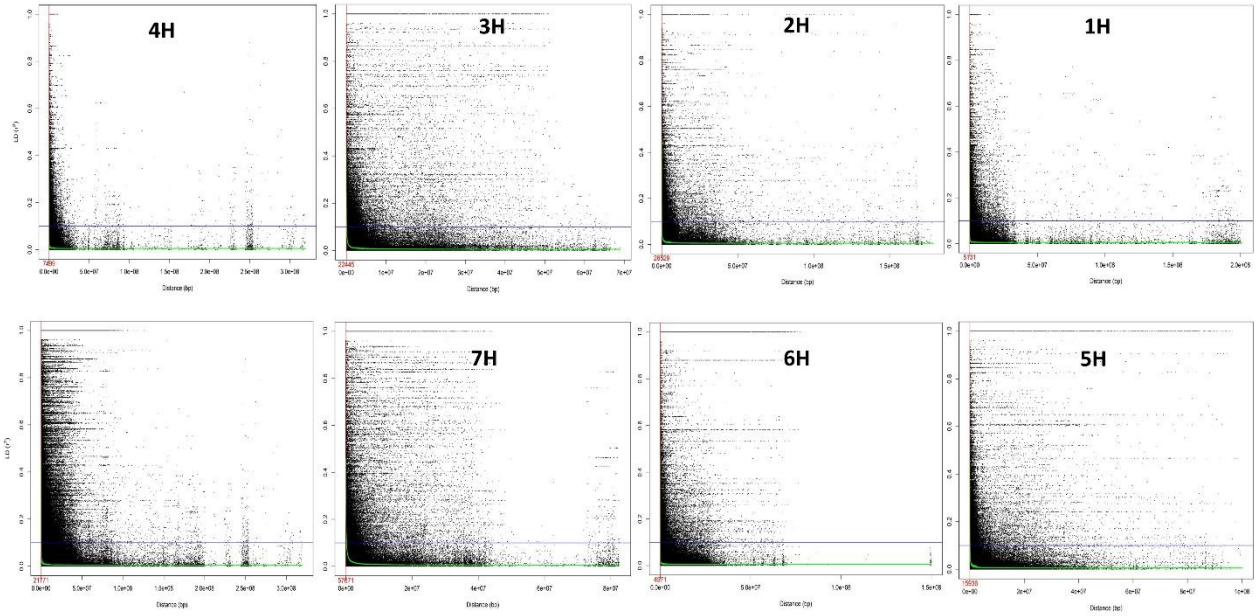


Figure IV.4. Linkage disequilibrium of *Hordeum spontaneum* across the different chromosomes and on the whole genome.

The mean R^2 values for the whole genome decreased with increasing pairwise distance. In general, LD showed a fast rate of decline, and the decay distance in the whole genome was approximately 0.02 Mb (Figure IV.4). The results highlight varying rates of LD decay across different chromosomes in wild barley. Chromosome 7H exhibits the highest LD decay value

(0.06), suggesting a slower rate of linkage disequilibrium decay compared to the other chromosomes, while chromosomes 1H, 4H, and 6H display faster decay rates (0.01, 0.01, and 0.004, respectively).

IV.3.5. Genetic diversity, differentiation and population structure

PCA was performed using the filtered set of 26,253 SNP markers to evaluate the genetic diversity of the 103 barley genotypes and showed a clear differentiation based on geographic origin and the sub-population identified by sNMF algorithm. The first axis of the PCA, explaining 9.2 % of the genotypic variance, clustered the genotypes according to their geographic origin and indicating that genotypes from the same geographic region tend to be more genetically similar. The genotypes from the North Africa are mostly clustered in the bottom part of the plot, whereas the genotypes from Central Asia and Middle East show some overlap, indicating closer genetic relationships between these populations (Figure IV.5.A). Furthermore, our finding revealed the genetic structure of the subpopulations, with clear distinctions between Sp1 and Sp2, and the presence of genetic admixture. Admixed genotypes are mostly located between the clusters of Sp1 and Sp2. This suggests that these gene have genetic contributions from both subpopulations. Their distribution also spans a broader area on the plot, indicating varied levels of admixture. However, genotypes classified under Sp1 appear to be most prevalent in North Africa, particularly in Libya (LYB), which could indicate that this subpopulation has a strong regional presence or historical significance in North Africa. The subpopulation (Sp2) is well-represented in Central Asia, especially in Tajikistan (TJK) and Kazakhstan (KAZ), suggesting that Sp2 may have originated from or is predominantly found in these areas (Figure IV.5.B).

The separation of subpopulation (Sp1), subpopulation (Sp2), and subpopulation (Sp3) into distinct clusters implies that there are genetic differences. A large number of admixed accessions are noted in the Middle East. This could suggest a central role for this region in the genetic diversity of wild barley. Sp1 is prominently found in Libya (North Africa), which may indicate a region-specific variety or adaptation. Sp2 is present in smaller numbers across several regions, suggesting a more dispersed or less dominant variety. Sp3 shows a significant presence in Central Asia, especially Tajikistan (TJK), Kazakhstan (KAZ), and Pakistan (PAK), implying that wild barley population may be more adapted to the environmental conditions of Central Asia (Figure IV.5.C).

The Adm group is the most prevalent, with a particularly high number in the Middle East.

Likewise, the PCA plot indicated that there is some degree of separation between the groups, suggesting distinct genetic variation among different subpopulations (Sp1, Sp2, Sp3, Sp4) and the admixed group (Adm). The Adm group is the most prevalent, with a particularly high number in the Middle East. The subpopulation Sp4 showed a strong presence in Central Asian countries, especially Tajikistan (TJK), the subpopulation Sp3 is found exclusively in Libya (North Africa), confirming a possible regional adaptation or a historical evolution that has led to its prevalence there (Figure IV.5.D).

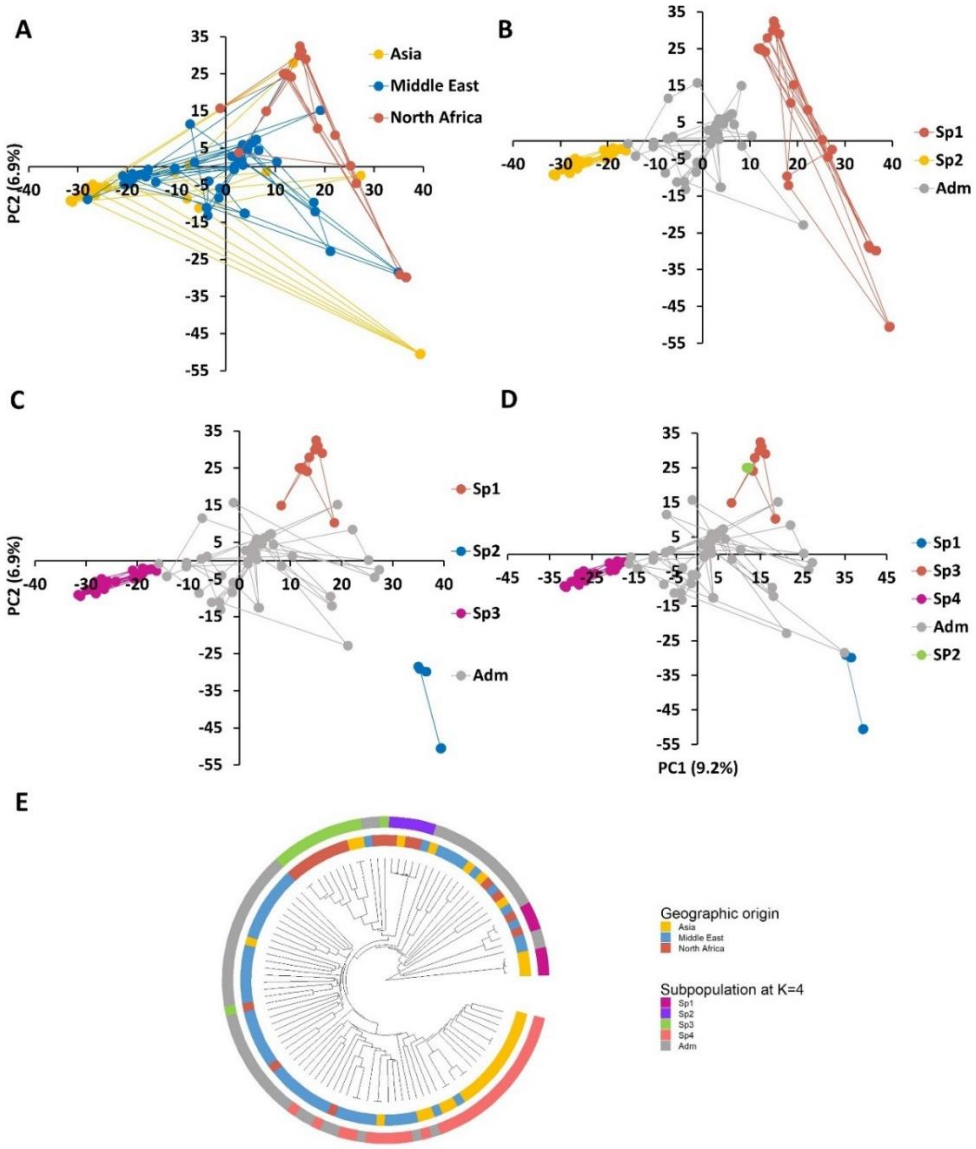


Figure IV.5. The genetic relationships and population structure of the 103 *Hordeum spontaneum* accessions using SNP markers inferred by PCA and sNMF analyses. Samples are colored according to geographic origin (A) and assignment to sNMF groups at K = 2 (B), K = 3 (C) and K=4 (D). Genotypes whose highest ancestry coefficient is less than 80% are colored

gray. The phylogenetic tree of the 103 *H. spontaneum* accessions based on 26253 SNPs (E). The 103 accessions are colored according to their geographic origin (inner circle) and to subpopulation (outer circle).

The genetic variation between and within groups was further explored through AMOVA tests and the analysis of genetic diversity parameters (Annex IV.1). AMOVA demonstrated that the geographic origin grouping accounted for more than 7.6 % of the whole genetic variation. However, the majority of variation comes from differences within geographic origins rather than between them. The results suggest that while geographic separation contributes to genetic differentiation, there is also substantial diversity within regions. In case of the subpopulation, AMOVA results indicated a significant proportion of the genetic variation in wild barley (23.06%) indicating that much of the variation is due to differences between subpopulations.
In the present study the genotypes from the Middle East have a significantly high number of private alleles (Npa) and Allelic Richness (Ar) with (3815) and (1.959) values, respectively. All regions show a difference between expected and observed heterozygosity, indicating potential population structure effect. Inbreeding Coefficient (FIS) values are high for all regions, especially in the Middle East (0.91) and Asia (0.98), indicating a high probability of inbreeding or a Wahlund effect, while North Africa has a lower FIS of 0.83. Furthermore, FST values provides insight into genetic differentiation between these populations. Our finding revealed that genotypes from the Middle East have the lowest FST value (0.055), suggesting less genetic differentiation compared to the other regions, and the genotypes from the North Africa shows the highest FST value (0.137), indicating the greatest genetic differentiation.

Table IV.3: Genetic parameters for each population are individualized by the geographic origin

Geographic Origin	N	Ar	Npa	He	Ho	FIS	FST
MiddleEast	54	1.959	3815	0.3	0.02	0.91	0.055
Asia	29	1.914	388	0.28	0	0.98	0.084
NorthAfrica	20	1.887	38	0.27	0.03	0.83	0.137

IV.3.6. Correlation of the genetic diversity metrics

The correlation matrix presents the pairwise correlations between Mean Minor Allele Frequency (Mean MAF), Mean Polymorphism Information Content (Mean PIC), Mean Heterozygosity, and LD decay. Our finding showed that a strong positive correlation between Mean MAF and Mean PIC (0.75), indicating that as the minor allele frequency increases, the polymorphism information content typically also increases. This suggests that genetic markers

are more informative in regions with higher minor allele frequencies. Mean PIC and LD decay also showed a high positive correlation of 0.80, which is the strongest correlation observed in this dataset. This indicates that chromosomes with more informative genetic markers tend to have a higher LD decay. Mean Heterozygosity and LD decay also have a moderate positive correlation of 0.66, implying that chromosomes with higher heterozygosity tend to have a greater LD decay. Furthermore, Mean Heterozygosity and Mean MAF show almost no correlation (-0.06), suggesting that the presence of different alleles at a particular locus is not associated with the frequency of the minor allele.

The heatmap suggests that there are significant positive correlations between most of the pairs of genetic diversity metrics, particularly between Mean PIC and LD decay, and between Mean MAF and Mean PIC. Mean Heterozygosity, while correlated with LD decay, shows weaker correlations with the other two metrics. This information is useful for understanding the genetic structure and diversity of the population in question.

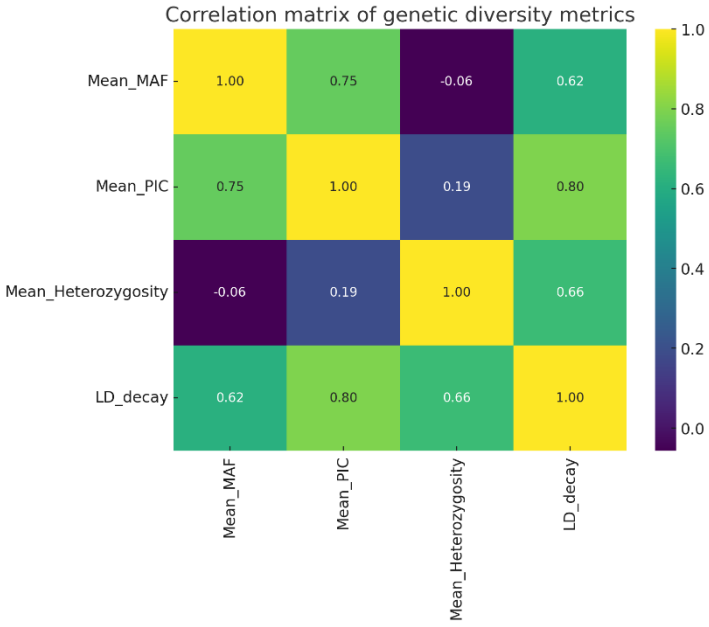


Figure IV.6. Correlation Matrix of Genetic Diversity Metrics in *Hordeum spontaneum* accessions

IV.3.7. Population structure of the wild barley

The genetic architecture of wild barley accessions was examined using sparse Non-negative Matrix Factorization (sNMF), cross-validation, and cross-entropy criterion methods. These analyses provided detailed insights into the genetic diversity within the population. The cross-entropy criterion showed a continuous decline with increasing cluster numbers (k), particularly between k=2 to k=20, indicating notable changes in population structure (Annex IV.2.A). At

k=2, nearly 43.69% of genotypes were identified as admixed, suggesting extensive genetic intermixing. Subpopulation 1, predominantly North African, constituted about 29.13%, while Subpopulation 2, mainly Central Asian, accounted for 27.18%. At k=3, almost half of the genotypes (49.51%) were admixed, with subpopulations 1, 2, and 3 representing 16.50%, 6.80%, and 27.18% of the genotypes, respectively. Moving to k=4, over half the genotypes (51.46%) remained admixed. The remaining genotypes were divided among smaller subpopulations: Sp1 (5.83%), Sp2 (4.85%), Sp3 (11.65%), and Sp4 (26.21%). This pattern illustrates a rich mosaic of genetic variation across the wild barley population.

IV.3.8. GWAS of scald resistance at the seedling stage

Performing GWAS for the assessment of resistance to the two *R. commune* isolates, SC-511, SC-1122 at the seedling stage, the best fitting model was the MLM procedure using PCA which accounted for population structure (Q) and relatedness (K).

At seedling stages, the genome scan identified 24 MTAs for both isolates (Figure IV.7). For isolate SRT-SC-1122, 18 MTAs were detected for across the seven-barley chromosomes except 5H chromosome, with a range of marker R^2 from 10.30 to 17.68 %, and additive effects ranging from -2.68 to 1.80. The highest phenotypic variation of 17.68 % was explained by the SNP marker SCRI_RS_127822 associated with Msc.SRT1 on 3H (10762816 bp) reducing the disease severity by -2.269, and by the SNP marker JHI-Hv50k-2016-147336 associated with Msc.SRT2 on 2H (7.66E+08 bp), reducing the disease severity by -0.1724 and explaining 15.265 % of phenotypic variation. However, the highest reduction in disease severity of -2.68 was detected on chromosome 2H, and explaining a phenotypic variation of 14.12%. For Rc isolate SRT-SC-511, about 6 MTAs were detected on 2H, 3H, and 7H chromosomes, with a range of marker R^2 from 10.35 to 18.69 %, and additive effects ranging from -3.33 to 2.48. The highest phenotypic variation of 18.69% was explained by the JHI-Hv50k-2016-115931 SNP marker associated with Msc.SRT19 on 2H (694692374 bp). The highest reduction in disease severity of -3.33 was caused by a SNP marker JHI-Hv50k-2016-513099 detected on chromosome 7H (642670653 bp), explaining a phenotypic variation of 14.56% (Annex IV.3, Figure IV.8).

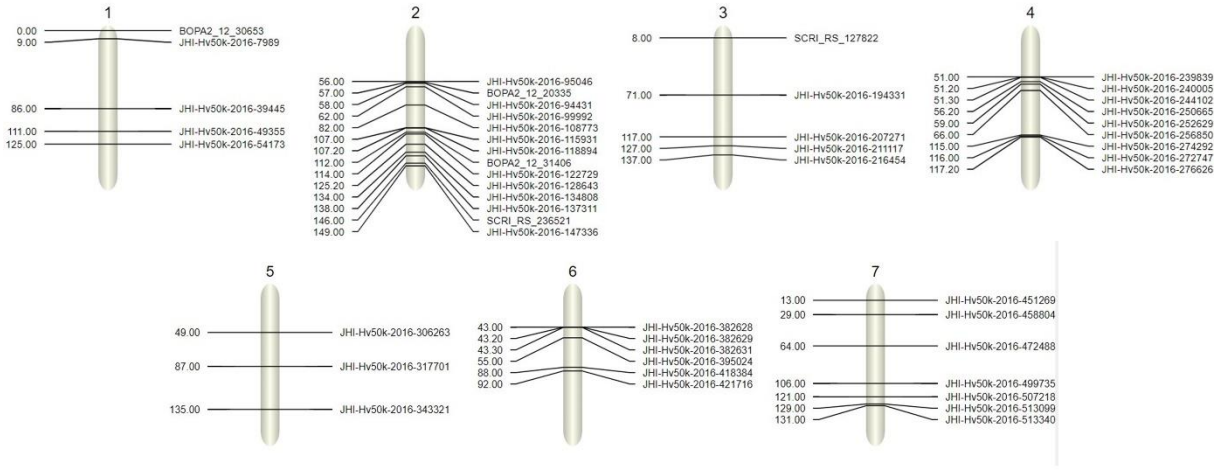


Figure IV.7. Genetic linkage map of significant SNP markers associated with seedling (SRT) and the adult plant stage (APS) resistance to scald. Markers are shown on the right and genetic distances (cM) are shown on the left.

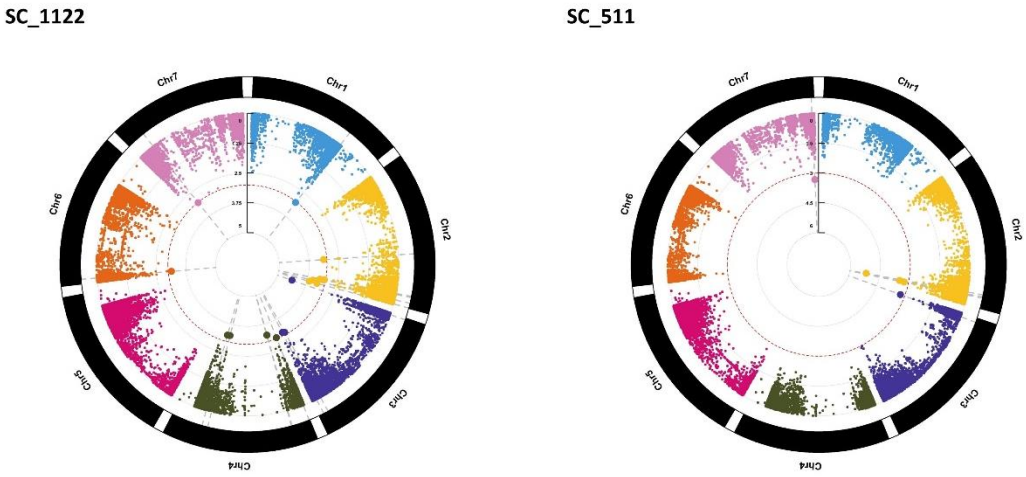


Figure IV.8. Genome-wide association mapping of *Hordeum spontaneum* scald resistance at the seedling stage for the SC-511 and SC-1122 isolates of *Rhynchosporium commune*

IV.3.9. GWAS of scald resistance at the adult stage

For the resistance at the adult plant stage, MLM procedure accounting for population structure (Q or PCA) and relatedness (K) was identified as the best fitting model. Our genome scan identified 31 markers trait associated to scald disease resistance at the adult plant growth stage across the two field locations. About 14 MTAs were detected at APR-Guich scattered on all barley chromosomes except 3H, with an additive effect ranging from -4.49 to 3.75, and a marker R^2 from 17.89- 10.14 %. The JHI-Hv50k-2016-451269 was the highest peak SNP marker detected on chromosome 7H (16899719 bp) and explained about 12.79% of phenotypic variation and reducing the disease severity by -3.09. Furthermore, the marker JHI-Hv50k-2016-

244102 was the second highest SNP marker were detected on the 4 H chromosome (458771700 bp), reducing the disease severity by -3.36 and explained about 12.57 % of the phenotypic variation. In case of MCH site, about 17 MTAs involved in scald disease resistance were detected across all barley chromosomes, with an additive effect of -6.96 to 4.84 and a range of marker R^2 of 9.92 to 19.25%. The highest reduction in disease severity of -6.96 was attributed to a SNP marker JHI-Hv50k-2016-137311 detected on chromosome 2H (745069584bp), explaining a phenotypic variation of 13.13 %. However, the highest phenotypic variation of 19.25% was explained by a SNP marker JHI-Hv50k-2016-458804 detected on the 7H chromosome (35194798 bp), and reducing the disease severity by 0.62. (Annex IV.4).

IV.3.10. Identification and in-silico expression analysis of putative candidate genes

The majority of the Marker-Trait Associations (MTAs) identified in the barley reference disease resistance and defense responses. BLAST search results have demonstrated their similarity to functional proteins/enzymes known to be involved in the disease resistance of the host plant, such as growth-regulating factor, Serine/threonine-protein kinase Receptor-like protein kinase family protein, glutamate receptor, phosphatidylinositol-4-phosphate 5-kinase family protein, glucan endo-1,3-beta-glucosidase GI, phospholipase D and progesterone 5 beta reductase (Annex IV.5 and Annex IV.6). In-silico gene expression analysis of these genes from BarleyExp database showed that majority of these genes were found to be differentially expressed across different tissues in barley. (Figure IV.9).

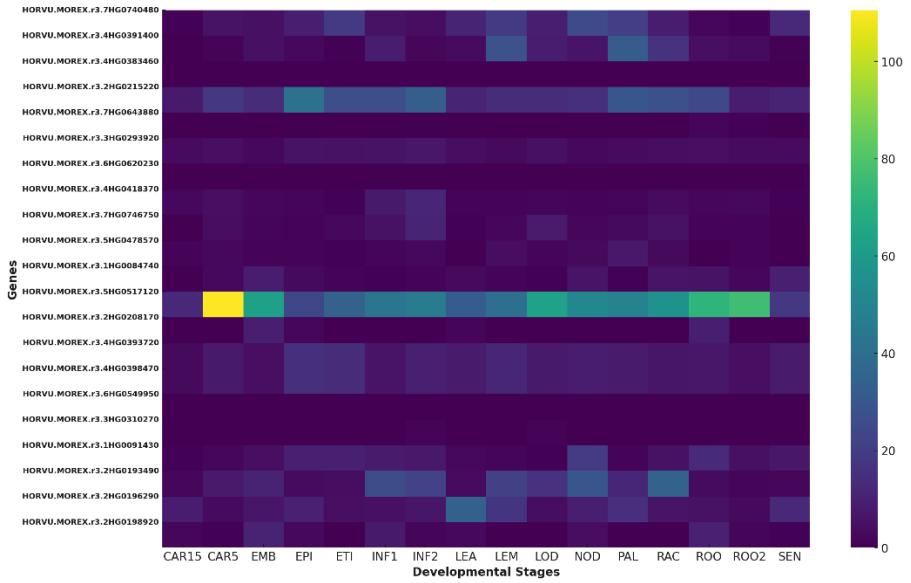


Figure IV.9. Expression profiles of candidate genes obtained in 103 barley accessions at different developmental stages of barley

IV.4 Discussion

Molecular markers have revolutionized the field of plant genetics and breeding by providing a means to visualize and quantify genetic variation at the DNA level. These markers serve as landmarks in the genome and can be associated with important traits, such as disease resistance, drought tolerance, or yield. They are indispensable for modern plant breeding program and for plant improvement that leverages genetic diversity as a resource for addressing the challenges of food security and agricultural sustainability in the face of global change (Savadi et al., 2021). Furthermore, SNP markers are extensively utilized in research exploring genetic diversity and evolutionary patterns (Comadran et al., 2011; Morrell & Clegg, 2007). In this research, among 36844 scorable Single Nucleotide Polymorphisms (SNPs) markers from the 50k iSelect SNP array (Bayer et al., 2017), a total of 26,253 exhibited polymorphism within the wild barley accessions used. The lowest number of SNPs was detected on chromosome 1H, while the highest number of SNPs was detected on chromosomes 5H and 2H, respectively as also reported by Bayer et al., (2017) and Bouhlal et al., (2022).

Genetic diversity was assessed using PIC value and the number of unique or private alleles. Private alleles are a critical tool in conservation genetics, as they offer a window into the evolutionary past of populations and measure the extent of the gene flow across populations (Barton & Slatkin, 1986; Kalinowski, 2004). The discovery of unique alleles plays a critical role in breeding for resistance. Furthermore, germplasm that harbors a larger array of unique alleles constitutes a valuable repository of novel genetic variants for incorporation into resistance breeding initiatives (Maanju et al., 2023). In addition, the percentage of private alleles detected in our study, is substantially higher at approximately 89.96% in the wild barley from the middle east region compared to the findings reported by Tanto-Hadado et al. (2010), where private alleles constituted only 23% of the total alleles. Previous studies have consistently identified a significantly higher number of unique alleles in wild barley when compared to its cultivated counterparts (Gong et al., 2009; Matus & Hayes, 2002). The discovery of a higher number of unique alleles in wild barley is a significant finding and are key indicators of genetic diversity in this germplasm and its potential as a valuable resource for crop improvement (Gong et al., 2009).

Botstein et al. (1980) established the Polymorphic Information Content (PIC) as a measure of genetic variation, with wild barley showing a moderate PIC value of 0.3, indicating modest diversity within the population, slightly lower than the 0.379 reported by Dai et al. (2012). The observed heterozygosity in the populations was low, typical for self-pollinating species like *H. spontaneum* (Hübner et al., 2009; Morrell et al., 2005; Thormann et al., 2016). While all regions

showed significant allelic richness, indicating a high genetic diversity, the Middle East emerged as the most genetically rich region, consistent with its role as centers for barley origin and domestication (Morrell & Clegg., 2007). Variations in allelic richness across regions may reflect historical genetic bottlenecks, gene flow, or selective pressures, with the Middle East's diversity potentially amplified by higher admixture rates (Hübner et al., 2009; Thormann et al., 2016).

Our study reveals a strong positive correlation (0.75) between Mean Minor Allele Frequency (Mean MAF) and Mean Polymorphism Information Content (Mean PIC), indicating that genomic areas with more minor alleles have more informative markers due to increased genetic variability. The correlations of LD decay with Mean MAF (0.62) and with Mean PIC (0.80) suggest that these good predictors of LD decay rates, with higher values indicating regions of higher genetic diversity and faster LD breakdown. This positive correlation with Mean PIC and LD decay contrasts the findings from studies of Gregory Berger et al. (2013) and Novakazi et al. (2020), possibly due to different population genetics and evolutionary dynamics. Interestingly, Mean Heterozygosity shows a moderate positive correlation with LD decay (0.66) but almost no correlation with Mean MAF and only a weak correlation with Mean PIC. This could suggest that the heterozygosity of the population is influenced by factors other than just the frequency of minor alleles or the informativeness of markers, such as mating patterns, population structure, or natural selection. The very weak negative correlation between Mean MAF and Mean Heterozygosity (-0.06) might appear counterintuitive since we might expect regions with a higher frequency of minor alleles to also have a higher proportion of heterozygous individuals. However, this lack of correlation might be due to a variety of factors, including the specific evolutionary history of each chromosome, the presence of selection pressures, or the effect of genetic drift. The weak correlation between Mean PIC and Mean Heterozygosity (0.19) could be attributed to the fact that PIC is a measure that considers not only the number of alleles but also the balance of their frequencies. Therefore, a marker can be highly informative (high PIC) even in a population with low heterozygosity if the allele frequencies are balanced.

Linkage disequilibrium

Genome-wide studies on barley populations have focused on linkage disequilibrium (LD) patterns which are critical for genetic study designs and breeding strategies. Variability in LD decay highlights the genomic complexity of wild barley (Bengtsson et al., 2017; Malysheva-Otto et al., 2006; Pasam et al., 2012). Notably, chromosome 7H shows higher LD decay,

indicative of slower LD breakdown and possibly lower recombination rates or selection pressures (Rostoks et al., 2006). Chromosome 6H exhibited the lowest LD decay at 0.004 Mb, suggesting a rapid decay and possibly higher recombination rates or a distinct evolutionary history. This low LD decay, suggesting frequent allele shuffling, makes it a prime candidate for precision trait mapping and understanding the recombination landscape (Flint-Garcia et al., 2003). The low LD decay on chromosome 6H suggests its potential for precise trait mapping and inheritance studies. This makes it valuable for locating quantitative trait loci (QTLs) for agronomic traits. Würschum (2012) highlights the importance of using both high and low LD regions for more effective genetic improvement strategies. Our study, confirmed the findings by Morrell et al. (2005), observed unexpectedly low linkage disequilibrium (LD) in the highly self-fertilizing wild barley (*Hordeum vulgare ssp. spontaneum*), contrary to typical inbreeding expectations. This suggests that despite a high selfing rate, wild barley maintains lower LD levels, possibly due to long-term genetic dynamics, such as historical outcrossing and prolonged evolutionary periods allowing occasional recombination to impact genetic structure (Lin et al., 2002; Nordborg & Bergelson., 1999). These rare recombination events, over extensive timescales, might break up linkage blocks, indicating that genetic diversity in inbred species can persist and evolve due to the cumulative effects of infrequent genetic exchanges and the species' long history (Morrell et al., 2005; Nordborg & Bergelson., 1999). Our study found that wild barley's 0.02 Mb LD decay reflects its high genetic diversity and rapid recombination, unshaped by the selection in cultivated varieties, confirming it as a rich reservoir of genes for the improvement of cultivated barley. The low LD may result from historical changes in mating systems and genetic recombination mechanisms (Charlesworth et al., 1997; Grant., 1958; Lin et al., 2002).

Population structure and differentiation

The application of molecular markers in population structure and phylogenetic analysis offers a detailed picture of the genetic architecture and evolutionary lineage of plant germplasm collections. This understanding is crucial for the effective conservation of genetic resources and their utilization in breeding programs (Sánchez-González et al., 2020). Our study reveals the complex genetic architecture of wild barley through methodologies like sparse Non-negative Matrix Factorization and cross-entropy criteria, showing a non-linear decline in cross-entropy with increased clustering and substantial shifts in population structure. This suggests a complex, non-uniform genetic stratification influenced by geographic origin, as indicated by Comadran et al. (2012) and Glaszmann et al. (2010).

The findings from the population structure, principal component analysis (PCA), and neighbor-joining clustering analysis revealed a differentiation among the accessions, categorized by their geographic origin. However, it is important to note that PCA often does not distinctly group accessions into separate clusters. This particularly holds true when the accessions are a mix of genetic backgrounds or come from diverse geographical locations, as detailed in the study by (Glaszmann et al., 2010). At higher ks, the categorization of a significant majority (51.46%) of the wild barley genotypes as admixed is a key finding, indicating extensive genetic diversity within the population. This diversity, characterized by a high degree of genetic sharing across subpopulations, suggests a dynamic intermixing of genetic material over time. For the admixed group (Adm), which is the most genetically diverse group, the dominant presence is from the Middle East. A significant portion of the genotypes, especially from the Middle East, is categorized as admixed, reflecting extensive genetic diversity and intermixing, possibly due to historical, ecological, and human-mediated factors (Abdel-Ghani et al., 2004; Hübner et al., 2012; Ellstrand et al., 2003; Nevo et al., 1986; Witcombe et al., 1982). Considerable genetic admixture was reported in the same geographic region that we have studied (Hübner et al., 2009; Thormann, Reeves, Reilley, Engels, et al., 2016). The distinct regional dominance within each subpopulation underscores the importance of considering geographical and environmental factors when studying genetic diversity. Our study's AMOVA analysis indicates that variation within subpopulations defined by population structure is more significant than that among geographic origins, contrasting the findings of Bouhlal et al. (2022b) and Jilal et al (2008). In addition, while geographic separation does contribute to genetic variation in wild barley, local factors within geographic origins play a much larger role. Conservation efforts should therefore not only consider the preservation of geographically distinct populations but also the maintenance of genetic diversity within these populations.

Identification of genomic regions associated to scald disease resistance

The exploration of genetic determinants underlying scald resistance in wild barley has been extended in our current genome-wide association study (GWAS). This study identified several MTAs on chromosome 1H that may play a role in conferring resistance to scald. Notably, these associations are distinct from the Rrs14 resistance gene previously mapped at 2.8 Mb and from the APR QTL qSc.APR9 located between 4.89-5.0 Mb on the short arm of 1H, which are in proximity to the introgressed Rrs14 locus from *Hordeum spontaneum* (Garvin et al., 2000).

The M.sc.APR13 associated to scald resistance at Guich with the peak marker (JHI-Hv50k-2016-39445), located at 504.07Mb, is positioned significantly away from the previously

identified qSc.APR9 and qSc.APR3 loci, suggesting the potential for a novel resistance mechanism. This substantial genomic distance from known resistance QTLs underscores the genetic diversity present within wild barley populations and hints at the existence of previously unidentified genetic resources that could be exploited for scald resistance. Notably, the marker BOPA2_12_30653 associated to MCH resistance at a mere 0.272697 Mb is intriguingly positioned at the chromosomal extremity, raising questions about its role in the genetic architecture of scald resistance. This genomic region challenges our current understanding of the genomic distribution of scald resistance genes and suggests a more complex genetic architecture than previously thought.

The M.sc.APR20 associated to scald resistance at APR-MCH with the peak SNP marker JHI-Hv50k-2016-7989 is not novel due to its proximity to Rrs-1H-1-4 reported by Yun et al. In (2005). However, 4 MTAs are considered novel, as they do not overlap with the known genomic region associated with scald disease resistance.

On chromosome 2H, a noteworthy proximity was observed of M.sc.APR3 with the peak marker JHI-Hv50k-2016-108773 associated to scald resistance at Guich site with a physical position at 658.629474 Mb which is situated approximately 3.52 Mb from QRrs2H.3 at 655.112232 Mb. and QTL QRrs2H.3 reported by Clare et al. (2023). The MTA M.sc.APR11 with the peak marker SCRI_RS_236521 associated to Guich resistance and located at 658.629474 Mb is of particular interest. Notably, the marker SCRI_RS_236521 is proximal to multiple known QTLs. The proximity of SCRI_RS_236521 to QA2 is particularly noteworthy at distance of roughly 2.03 Mb (Looseley et al. (2018), and to QTLCW2H.1 and QTLCW2H.2 at 664.3 Mb (~5.67 Mb distance) (Looseley et al., 2012), in addition to other QTLS QTLSR2H.1 and QTLSR2H.2, which were previously reported at 664.9 Mb (~6.27 Mb distance) (Looseley et al., 2014) and to QTLG2H at 669.2 Mb (~10.57 Mb distance) (Gawenda et al., 2015). The locus qScSRT2, identified in previous study as a significant QTL for scald resistance in barley, lies within the genomic region spanning 579.22-583.15 Mb on chromosome 2H. The proximity of the M.sc.APR1, with the SNP marker JHI-Hv50k-2016-99992 located at 589.512031 Mb, suggests that these two genetic markers overlap within the same broad region on the chromosome 2H.

Msc.SRT13. associated to the SRT-SC-1122 isolate resistance with the SNP marker JHI-Hv50k-2016-94431was closeley reported to the QTL Qsc2H.2-Seebe by Zantinge et al in (2019). Whereas, 11 MTAs were considered as novel.

On chromosome 3H, the M.sc.APR16 identified with the SNP marker JHI-Hv50k-2016-194331 at 571.30548 Mb associated to scald resistance at MCH and was previously reported in four previous studies, one of which noted the close proximity to vrs1 (Li and Zhou., 2011; Looseley

et al., 2018; Fériani et al., 2020; clare et al., 2023). Similarly, the Msc.SRT11, with the JHI-Hv50k-2016-216454 marker at 670.445657 Mb was found to be in proximity to the QTL "QRrs-3H.4" reported by (Clare et al., 2023). However, 4 novel MTAs considered as novel and explaining a substantial proportion of the phenotypic variance, suggesting that these markers are closely linked to the genes or genomic regions conferring resistance to scald.

On chromosome 4H, QTLHT4H at 576.3 Mb QTL has been previously reported by Spaner et al. (1998), and identified as a region associated with scald resistance. The Msc.SRT9 associated with SNP Marker JHI-Hv50k-2016-256850 is in close proximity to this QTL.

The Msc.SRT17 associated to the SRT-SC-1122 resistance with the peak marker JHI-Hv50k-2016-228197 at 5.37 is in close proximity to multiple QTLs including "QSc.VlWa.4H.1" (Wang et al., 2014), "Rrs16 Hb2", "Rrs16 Hb1", "QSc.VlWa.4H.2" (Pickering et al., 2006), "QTLVB4H.1", "QTLVB4H.3*" (Wallwork et al., 2014), and QRrs-4H.1 (Clare et al., 2023), indicating a rich region for scald resistance. Another, Msc.SRT6 MTA, conferring resistance to SRT-SC-1122 isolate with SNP marker JHI-Hv50k-2016-239839 is about 0.31 Mb from "QRh.S42-4H.a", reported by von Korff et al. (2005), suggesting a strong potential linkage to this QTL. Further, the Msc.SRT7 with SNP marker JHI-Hv50k-2016-239957 is roughly 2.20 Mb from QRh.S42-4H.a (von Korff et al., 2005), Msc.SRT8 with SNP marker "JHI-Hv50k-2016-240005" is around 2.82 Mb from "QRh.S42-4H.a" (von Korff et al., 2005). Interestingly, the M.sc.APR10 with SNP marker JHI-Hv50k-2016-244102 is coincides with two prevouisely QTLS "QTLVB4H.2*" and "QTLVB4H.4*" reported by Wallwork et al. (2014). Hiddar et in 2023 map an APR QTL qSc.APR18 which may represent the same QTL M.sc.APR2 find in our study controlling resistance at the same location at Guich station.

In addition, the Msc.SRT5 with the SNP marker JHI-Hv50k-2016-252629 is not novel due its proximity to the QTL qSc.APR5 reported previously by (Hiddar et al., 2023). Furthermore, the two MTAs M.sc.APR14 and M.sc.APR18 confering the scald disease resistance at Guich and MCH stations respectively, these markers are linked to genomic regions already known to influence scald resistance which coincide with the same QTL reported by (Hiddar et al., 2023). However 1 MTA on chromosome 4H M.sc.APR8 is considered novel.

On chromosome 5H, none of the MTAs detected on this chromosome M.sc.APR4, M.sc.APR24, and M.sc.APR30 are novel. The MTA M.sc.APR4 at 553.25 Mb was detected and coincided with the same genomic region associated with scald resistance QTLs reported by Hiddar et al., (2023) and Looseley et al. (2012). Two MTAs M.sc.APR30 and M.sc.APR24 controlling scald resistance at MCH station were detected in the same genomic regions, with

M.sc.APR4 at 553.25 Mb is closest to QTLs qSc.APR21 found by Hiddar et al in (2023), and QRrs-5H.1 reported by (Clrae et al., 2023). Furthermore, the MTA M.sc.APR4 detected at adult plant stage conferring resistance at Guihc station with the SNP marker JHI-Hv50k-2016-317701 is not novel as it is close to qSc.APR39 located at 553.49 Mb, reported by (Hiddar et al. 2023).

Chromosome 6H, possesses a major gene named Rrs13, originally mapped in an interspecific cross with *H. spontaneum* (Abbott et al., 1995; Genger et al., 2003a), and also detected conferring resistance at adult plant stage by Spaner et al., (1998), Shtaya et al., (2006) and (Hiddar et al., 2023). Likewise, we detected one MTA linked to Msc.SRT12 conferring resistance to SC-1122 isolate with the peak SNP marker JHI-Hv50k-2016-379923 on 25.6 Mb. Another MTA linked to M.sc.APR6 controlling resistance at adult plant stage and mapped at 555.7 is previously identified and mapped in the same region by Looseley et al. (2012), and coincide with the previously qSc.APR42 map by (Hiddar et al., 2023). A M.sc.APR17 detected at MCH site and mapped at 545.7 Mb coincides with the Qsc3.6H.7-Seebe reported by Zantinge et al., (2019). However about 3 MTAs (M.sc.APR19, M.sc.APR23, and M.sc.APR28) potentially considered novel.

Seven MTAs were mapped on chromosome 7H and constituted 3 MTAs validated by our study. The SNP Marker JHI-Hv50k-2016-451269 associated to resistance at Guich site was detected in close proximity of Rrs12, which is the major scald resistance gene which is introgressed from *H. spontaneum* and mapped at 16.0 Mb on chromosome 7H (Abbott et al., 1992). Further, another MTA associated to Guich resistance, with the SNP Marker JHI-Hv50k-2016-499735 was detected and mapped at 616.3 Mb may represent the same QTL reported in previous studies (Hiddar et al., 2023; Wang et al. 2014; Thauvin et al. 2022). In adition the M.sc.APR15 with the SNP marker is coincide with the QTL QA8 at 35.5 Mb, reported by Looseley et al. (2018). About 4 MTAs that are potentially novel on chromosome 7H.

Understanding host-pathogen interactions at the genetic level is vital for identifying scald resistance in plants. Plants first defend against pathogens through pattern recognition receptors (PRRs) that detect microbe-associated molecular patterns, as noted by Miya et al. (2007). These receptors, often with a leucine-rich-repeat (LRR) or chitin-binding (LysM) domain, can initiate defense mechanisms. However, pathogens may evade these defenses using effectors, against which plants deploy a second defense layer through nucleotide-binding leucine-rich repeat (NLR) receptors, leading to effector-triggered immunity (ETI), as described by Jones and Dangl

(2006). In barley, *R. commune* interactions and specific proteins like NIP1, NIP2, and NIP3 play roles in this complex defense, with studies by Kirsten et al. (2012) and Schürch et al. (2004) highlighting the variability and evolution of these pathogen strategies. The complexity of interactions and rapid evolution of pathogens like *R. commune* necessitate the identification and deployment of both qualitative and quantitative resistance genes, as argued by Stukenbrock and McDonald (2009) and Mohd-Assaad et al. (2019). Barley genome and predicted protein repertoire availability will facilitate candidate gene identification using functional homology-based BLAST searches with marker sequences linked to mapped QTL.

Glutamate receptor plant play a crucial role in defense mechanisms as reported by Feng Li et al., (2013), the sequence of the BOPA1_14832-296 SNP marker (704890469 bp) on chromosome 2H encode this protein (HORVU.MOREX.r3.2HG0196290). A SNP marker JHI-Hv50k-2016-115931 associated with the msc.SRT19 on chromosome 2H (694692374) encodes growth-regulating factor (HORVU.MOREX.r3.2HG0193490), which play an important role in biotic stress- as reported by several studies (Hewezi et al., 2012; Liu et al., 2014). Further, on chromosome 2H, the SNP marker JHI-Hv50k-2016-122729 encodes phosphatidylinositol 4-phosphate 5-kinase (PIP5K) (HORVU.MOREX.r3.2HG0198920). Recent studies have shed light on the involvement of phosphatidylinositol 4-phosphate 5-kinase (PIP5K) genes in plant responses to biotic stress, particularly in defense against pathogens. Menzel et al. (2019) reported that the activity of the PIP5K6 enzyme is downregulated by the signaling pathways activated by pathogen-associated molecular patterns (PAMPs) via a Mitogen-Activated Protein Kinase (MAPK) cascade. This suggests that upon pathogen detection, plants can modulate lipid signaling as part of their defense strategy. Further, research by Qin et al. (2020) has revealed that *Arabidopsis* mutants deficient in two PIP5K genes, pip5k1 and pip5k2, show reduced levels of the lipid PtdInsP2, which in turn inhibits the development of fungal pathogen. On the chromosome 3H, the SNP marker JHI-Hv50k-2016-211117 (658334577 bp), encodes methyl esterase (HORVU3Hr1G098360), which plays a crucial role in the plant's immune response (Jerome Pelloux et al., 2007). On chromosome 7H, the SNP marker JHI-Hv50k-2016-513099 (642670653 bp), encode Vacuolar membrane protease (HORVU7Hr1G116080), which plays an essential role in programmed cell death (PCD). The study found that tobacco plants deficient in VPE were unable to execute virus-induced hypersensitive cell death, highlighting the enzyme's crucial role in this form of plant PCD (Noriyukihatsugai et al., 2004). Research has consistently shown that the production of glucan endo-1,3-beta-glucosidase in plants is induced by infections from a variety of pathogens, including fungi, bacteria, and viruses (Atnafu and Mulugeta., 2021). As a response to infection, plants significantly increase the enzyme's

concentration. For example, elevated levels of mRNA encoding for glucan endo-1,3-beta-glucosidase have been observed in tomato leaves affected by *Cladosporium fulvum* (Beerhues L et al., 1994), barley attacked by powdery mildew (Ignatius SM et al., 1994). A SNP marker JHI-Hv50k-2016-216454 is associated with a Msc.SRT11 on chromosome 3H at (670445657bp) encodes a glucan endo-1,3-beta-glucosidase (HORVU3Hr1G106140). Further, the phospholipase D P (HORVU6Hr1G012780), it also known for its significant role in plant defense against the pathogen attack (Kalachova et al., 2013). The JHI-Hv50k-2016-379923 on chromosome 6H (25291141bp), associated with Msc.SRT12 encodes the phospholipase D (HORVU6Hr1G012780). Progesterone 5 beta reductase (P5βR) is involved in the biosynthesis of specific terpenes and iridoid compounds, which are important in plant defense (Kristin Rudolph et al., 2016). The SNP marker JHI-Hv50k-2016-472488 associated with Msc.SRT18 on chromosome 7 (98424042 bp), encodes Progesterone 5 beta reductase (P5βR) (HORVU7Hr1G038590). The SNP marker JHI-Hv50k-2016-147342 (765723329 bp), with a Msc.SRT4n on chromosome 2H encode peptide transporter which also plays a role in host–pathogen interaction.

The phytotoxin phaseolotoxin is among the many di- and tripeptides that the *Arabidopsis* AtPTR1 peptide transporter participates in transporting, suggesting its function in pathogen interaction. Its ability to transport various compounds originating from pathogens raises the possibility that it regulates the entry and effects of these compounds on plant cells, hence playing a role in pathogen defense systems (Dietrich et al., 2004). In plants, Histones Acetyltransferases are integral in regulating gene expression in response to various stresses including biotic stress as reviewed by (Boycheva et al., 2014),the sequence of the JHI-Hv50k-2016-418387 SNP marker (545727109 bp) on chromosome 6H encode this protein (HORVU.MOREX.r3.6HG0620230).

Several SNP markers encoding functional proteins that have been involved in plant defense against several fungal infections have been detected by our study. Breeders can use the newly identified QTLs in their barley breeding programs, and they can use the information for fine mapping, marker assisted selection, and gene cloning to boost resistance to scald disease. The genetic diversity of wild barley offers an opportunity for the development of new barley cultivars with higher scald disease resistance, promoting more resilient and sustainable farming methods. The safeguarding of crucial crops like barley against new threats will depend more and more on the conservation and use of genetic resources like those discovered in wild barley as worldwide agricultural issues continue to change.

IV.5 Conclusion

This investigation into the genetic resources of *Hordeum vulgare* subsp. *spontaneum* (wild barley) has shed light on its potential in enhancing resistance against *Rhynchosporium commune*, the causative agent of scald disease in barley. Harnessing this genetic potential not only contributes to securing barley crops against this pathogen but also supports broader goals of sustainable and resilient agricultural systems. Pre-breeding efforts need to be strengthened to introgress useful genes from *H. spontaneum* to develop high yielding and disease resistant varieties which will cope with the rapid changing of virulence of scald populations.

Chapter V: General discussion

Barley is one of the most important field crops around the world, its recognition has grown worldwide due to its numerous health benefits and variety of uses. However, barley quality and productivity are affected by various phytopathogens. Barley scald disease, caused by the fungal pathogen *Rhynchosporium commune*, is a significant concern for barley crop worldwide. This disease poses a considerable threat to barley production and can lead to significant economic losses for farmers and the agriculture industry. The indiscriminate use of fungicides may lead to the development of resistant strains of the pathogen, highlighting the need for sustainable and environmentally friendly disease management strategies. Breeding for resistance is one of the most promising approaches to combat barley scald disease effectively. Developing and deploying resistant barley varieties can significantly reduce the disease's impact and reliance on chemical control measures and also remains the most appropriate disease control which requiring continuous search for new sources of resistance to overcome the new virulence of the pathogen population. The introduction, incorporation of the sources of resistance will require starting pre-breeding effort and the use of associated molecular markers for efficient selection of lines combining major and minor genes of resistance.

Four sets of germplasm were used which include (FIGS, GCP) and set of *hordeum spontaneum* accessions all supplies by ICARDA genebank and AM panel maintained by breeders to serve sources of parental material for their breeding program. The evaluation was done at the seedling stage using two isolates and at adult plant stage at two locations in Morocco and one location in Ethiopia. The material was subjected to genotyping using Illumina 50 k array to conduct GWAS to identify genomic regions associated with resistance. The results showed that all subsets showed sources of resistance to scald at both seedling and adult plant stages, but with different percentages. These differences can be attributed to differences in among isolates and among pathogen populations. Our study marks the first comparison of FIGS approach with another subset based on core collection, and the outcomes underscore the effectiveness of this methodology in exploring genebank collections. Notably, the identification of resistance sources within both the Focused Identification of Germplasm Strategy (FIGS) and the Generation Challenge Program (GCP) subsets highlights the potential of leveraging genebank collections for identifying valuable traits. The higher percentage of resistant accessions within the FIGS subset suggests the efficacy of this approach in targeting specific traits, such as scald resistance.

Breeders prefer the use of germplasm of the panel as they include improved parental and are reluctant to use landraces and wild relatives. However, the landraces and wild hordeum could contribute novel sources of resistance along with extending the genetic base of cultivated barley. Therefore, the conservation and the use of genetic resources is an important supporting activity to crop improvement. FIGS showed its relevance identifying larger number of resistances compared to the GCP. Generally, showed it efficiency of the approach developed to effectively mining the large collection. The FIGS approach can be further improved by using machine learning algorithms as shown by our results, the performance of predictive models varied depending on the environment at the adult plant stage, and on the isolates at the seedling stage. Four machine learning models were tuned on training sets to predict scald reactions on the test sets based on diverse metrics (accuracy, specificity, and Kappa). All models efficiently identified resistant accessions with specificities higher than 0.88 but showed different performances between isolates at the seedling and to field populations at the adult plant stage. The findings of our study will help in fine-tuning FIGS approach using machine learning for the selection of best-bet subsets for resistance to scald disease from the large number of gene-bank accessions.

Crop wild relatives, exclusively, *Hordeum vulgare ssp. spontaneum* is an important genetic resource needed to develop new adapted varieties. Our results showed that wild barley include sources of resistance to scald disease at both growth stages. As the field of genomics advances, the identification and utilization of associated molecular markers become increasingly precise and powerful, thus the development of associated molecular markers to introgress resistance loci to develop resistant varieties is crucial and streamlines the breeding process. To our knowledge, this is the first GWAS study of scald resistance in spring barley and wild barley from North Africa. The results from both AM and HS panels demonstrate the utility of GWAS in identifying both known and novel QTLs associated with disease resistance, which can be crucial for breeding programs aiming to enhance scald resistance in barley. For wild barley about 26,253 SNP markers underwent genetic analysis after quality control measures. In contrast, AM2017 used about 36,793 SNP markers for genetic analysis post-quality control. The higher number of SNP markers used in AM2017 indicates a possibly more complex genetic makeup of the cultivated barley that necessitates a denser marker coverage for accurate association mapping. The association mapping (AM) panel constructed by breeders has confirmed 18 QTL that were reported in previous studies. While, the *Hordeum spontaneum* has confirmed 27 QTL from previous studies, which is notably higher than the AM panel. The

differences in QTL verification, novel QTL discovery, number of SNP markers used, and GWAS results between AM2017 and HS can be primarily attributed to the genetic diversity and background of the plant materials. *Hordeum spontaneum*, as a wild relative of cultivated barley, harbors a rich reservoir of genetic variation, including for disease resistance traits, which is reflected in the larger number of verified and novel QTLs. On the other hand, AM2017, being a cultivated barley, might have gone through selective breeding that narrows down its genetic diversity but enhances certain desirable traits, including disease resistance.

Based on the results deployment of genes of resistance should utilize sequential genes or pyramiding genes in absence of the sexual stage. Durable resistance might require combining major genes with minor genes and molecular markers are crucial in this regard. These markers will allow also to consider extending the source of resistance to accessions with moderately resistant (MR) reaction. The results of this study have provided several sources of resistance and associated molecular markers which can be used in breeding varieties resistant to scald disease using marker assisted selection and genomic selection.

General Conclusion and perspectives

This research has identified sources of resistance to scald at seedling and adult plant stages in the various sets screened, but with different proportions. The subsets of the Focus Identification Germplasm Strategy (FIGS) and the Generation Challenge Program (GCP) identified sources of resistance in manageable sets of landrace accessions. Our findings have demonstrated the effectiveness of FIGS approach in identifying with a higher frequency resistant accession for scald disease in barley landraces compared to the GCP. By strategically filtering agro-climatic conditions favoring disease development, FIGS optimizes germplasm selection, increasing the likelihood of finding accessions with natural resistance traits. The success of FIGS in identifying resistant accessions highlights the importance of targeted approaches in genebanking and crop improvement. The FIGS maximizes the utilization of limited resources and facilitates the identification of valuable genetic resources for resistance breeding programs. Our findings highlight the need for more research on fine tuning FIGS approach by using the predictive models through the use of machine learning and by extending the models to include the diversity of virulence of scald populations and the associated molecular markers. By utilizing advanced computational methods of machine learning algorithms for disease resistance modeling are opened up new avenues better targeting of sources of resistance in genetic resources and efficient breeding strategies.

In addition, a high-throughput genotyping has transformed the landscape of genetic research by providing a powerful tool that can significantly accelerate the pace of scientific discovery by enabling the rapid and cost-effective analysis of thousands to millions of genetic markers. Our findings highlight the potential of the Genome-Wide Association Study (GWAS) in genetic dissection of scald resistance. Not only where several markers were found to be located within already known QTLs (Quantitative Trait Loci) associated with scald disease, but the study also detected novel QTLs. This discovery of new genomic regions linked to scald resistance opens up new possibilities for targeted breeding efforts.

Numerous QTLs were associated to scald disease resistance at seedling and adult plant growth stages, these associations encompassed 39 and 56 quantitative trait loci (QTLs), respectively, reflecting the complex nature of the genetic determinants underlying the resistance trait. This extensive set of associations demonstrates the multifaceted genetic architecture of scald resistance, suggesting that a combination of genes and pathways contribute to the plant's ability to fend off the fungal pathogen. The genomic distribution of these markers across all chromosomes, with a particular concentration on chromosomes 3H and 7H, underscores the

importance of understanding the spatial organization of genes in relation to disease resistance. This distribution pattern could potentially offer clues about the co-localization of functionally related genes or the presence of hotspots for genetic diversity that contribute to resistance.

Importantly, this study not only validated 45 previously reported QTL but also identify 36 novel sources of resistance and genomic regions that had previously remained undiscovered. This GWAS studies have provided significant insights into the genetic architecture of scald resistance in barley and demonstrated the potential for using modern genomics technologies and bioinformatics tools to advance crop improvement efforts.

The identification of diverse sources of resistance and the molecular tagging of new genomic regions associated with resistance to *R. commune* represents a significant step forward in the quest to enhance scald resistance in barley germplasm. This research has laid the foundation for the development of marker-assisted selection (MAS) and genomic selection strategies, which can facilitate the continuous improvement of barley varieties with enhanced scald resistance. However, achieving durable scald resistance requires further investigations and advancements. Understanding the dynamics of the *R. commune* pathogen population is crucial for implementing effective resistance strategies. This knowledge can help breeders anticipate potential shifts in pathogen virulence and develop resistant varieties that can withstand evolving pathogen strains.

Moreover, the efficient introgression of major and minor resistance genes using molecular markers is essential for enhancing scald resistance in barley germplasm. This involves the strategic transfer of genes conferring resistance from diverse sources including from accessions of wild *Hordeum* such as *H. spontaneum* into elite breeding lines to create robust and long-lasting resistance against the pathogen. Molecular markers can aid in accurately identifying and transferring these beneficial genes, thus accelerating the breeding process and reducing the reliance on time-consuming and labor-intensive conventional breeding methods. Continued research in this area, coupled with ongoing monitoring of pathogen dynamics, will undoubtedly contribute to the development of more robust and productive barley cultivars.

In barley breeding programs, breeders prefer to use the panels as most of genotypes were either elite lines or released varieties from different countries, which offers numerous advantages that contribute to the efficiency of the breeding process; however, it is crucial to use and to incorporate disease resistance genes from wild relatives and landraces into cultivated crops which offers a range of advantages that contribute to more resilient, sustainable, and adaptable agricultural systems. Furthermore, utilizing wild relatives in breeding programs contributes to

the conservation of biodiversity by ensuring that valuable genetic resources are not lost. By harnessing the genetic diversity present in these populations, breeders can develop crop varieties that can withstand the challenges posed by evolving pathogens and changing environmental conditions. However, more coordinated pre-breeding efforts are needed in the future to introgress valuable traits from the wild relative species.

Overall, addressing the challenges posed by barley scald disease requires collaborative efforts from plant pathologists, breeders, agronomists, and policymakers. By promoting sustainable agricultural practices, investing in research, and fostering global cooperation, we can work towards mitigating the impact of barley scald disease and ensuring food security for future generations.

Annexes

Annex I.1: Summary of scald resistance quantitative trait loci (QTLs) ordered by chromosome position giving logarithm of the odds (LOD) scores, percentage of phenotypic variation

Original QTL	**Chr**	**Physical position (Mb)**	**LOD**	**Closest or flanking markers**	**Resistant source**	**Reference**
QTLID1H*	1H	Unknown	-	-	Igri	Backes et al. (1995)
Rrs14	1H	2.8	-	Hor2	CPI 109829 (*Hordeum spontaneum*)	Backes et al. (1995)
Rrs-1H-1-4	1H	12.4	46.2	Bmac0144—Bmac0213	OUH602 (PI 682043) (*H. spontaneum*)	Yun et al. (2005)
QSc.YeFr-1H	1H	286.2	3.7	bPb-9337	Yerong	Li and Zhou (2011)
QTLTritonRrs2Hnatural	2H	10.4	13.4	GBM1281	Triton	Wagner ***et al***. (2008)
Rrs17 (Rrs15 (CI8288))	2H	10.4	49.6	GBM1281	Triton	Wagner ***et al***. (2008)
QTLCDPB2H.1*	2H	17.9	3.6	11_1175	SBCC145 (Cebada Del Pais	Hofmann et al. (2013)
QRh.S42-2H.a	2H	24.2	4.2	GBM1052	ISR42-8 (*H. spontaneum*)	von Korff et al. (2005)
QTLCB2H.2*	2H	28.2	2.8	11_1159	SBCC154 (Cebada)	Hofmann et al. (2013)
QTLIB2H.1	2H	303.5	2.6	ctaaca1—cttaca6	Abyssinian (CIho 668)	Grønnerød ***et al***. (2002)
QTLIB2H.2*	2H	303.5	2.8	ctaaca21—caaacg3	Abyssinian (CIho 668)	Grønnerød ***et al***. (2002)
Qsc2H.2-Seebe	2H	486.4	4.5	chr2H_543448499	Seebe	Zantinge ***et al***. (2019)
Qsc2H.1-Seebe	2H	486.4	4.5	chr2H_543512242	Seebe	Zantinge ***et al***. (2019)
QTLRS7a	2H	636.1	2.5	P71m293a—HVM54	WI2291	Sayed et al. (2004)
QSc.TxFr-2H	2H	651.7	2.9	bPb-7841	Franklin	Li and Zhou (2011)

QTLCW2H.[1]	2H	664.3	5.0	11_10791-11_10085	WB05-13 (Leonie/Pearl)	Looseley ***et al***. (2012)
QTLCW2H.2*	2H	664.3	4.0	11_10791-11_10085	WB05-13 (Leonie/Pearl)	Looseley ***et al***. (2012)
QTLSR2H.2*	2H	664.9	4.7	11_10791-11_10085	Saffron	Looseley ***et al***. (2014)
QTLSR2H.1*	2H	664.9	5.7	11_10791-11_10085	Saffron	Looseley ***et al***. (2014)
QTLID3H*	3H	Unknown	-	-	Igri	Backes et al. (1995)
QTLSR3H.1*	3H	13.5	4.6	11_21027—11_20968	Retriever	Looseley et al. (2014)
QTLRS5	3H	16.3	4.1	P294m34b-P18m184b	Tadmor	Sayed et al. (2004)
QTLRS4	3H	16.3	2.9	HVLTPPB—P71m82b	Tadmor	Sayed et al. (2004)
QTLHT3H*	3H	19.7	4.2	MWG2040	Harrington	Spaner et al. (1998
QSc.TxFr-3H	3H	32.3	5.2	bPb-7356	Franklin	Li et Zhou (2011)
QSc.YeFr-3H	3H	149.5	15.2	Bmag0006	Yerong	Li et Zhou (2011)
QTL-WAIYerong-3H	3H	149.5	38.7	Bmag0006	Yerong	Zhang et al. (2019)
QTLSR-3H-2016	3H	149.5	8.8	Bmag0006	Yerong	Zhang ***et al***. (2019)
QTLSR-3H-2017	3H	149.5	4.4	Bmag0006	Yerong	Zhang ***et al***. (2019)
qSUK7_3	3H	324.5	24.5	1_1342—2_1129	Steptoe (CIho 15229)	Coulter et al. (2019)
QTL-ICARDA2	3H	383.9	-	Xbmag603	ICARDA2	Genger ***et al***. (2003b
QTL-ICARDA4	3H	383.9	-	Xbmag603	ICARDA4	Genger ***et al***. (2003b
QTL-ICARDA9	3H	383.9	-	Xbmag603	ICARDA9	Genger ***et al***. (2003b
Rrs1 (Sultan)	3H	383.9	-	Xbmag603—Xbmag225	Sultan	Genger ***et al***. (2003b
QTLSR3H.4*	3H	428.8	12.4	11_11401—11_10728	Retriever	Looseley ***et al***. (2014)
QTLSR3H.2	3H	428.8	31.3	11_11401—11_10728	Retriever	Looseley ***et al***. (2014)
Rrs.B87	3H	442.7	-	bcd828	B87/14 (Selection from Pye S)	Williams et al. (2001)

QTLCDPB3H.2*	3H	446.7	73.4	11_0010	SBCC145 (Cebada Del Pais	Hofmann et al. (2013)
Rrs1 (Rh4)	3H	446.9	-	chr3H_ 490,253,069	CIho3515 (PI 58052)	Looseley ***et al***. (2020)
Rrs1 (Rh4)	3H	446.9	-	chr3H_ 490,253,069	SBCC154 (Cebada)	Looseley ***et al***. (2020)
Rrs1 (Rh4)	3H	446.9	-	chr3H_490,253,069	SBCC145 (Cebada Del Pais)	Looseley ***et al***. (2020)
Rrs1 (Rh4)	3H	446.9	-	chr3H_490,253,069	Retriever	Looseley ***et al***. (2020)
Rhy	3H	447.3	-	CDO1174	E224/3 (SCRI breeding line)	Barua et al. (1993)
QTLIS3H.2*	3H	447.3	2.7	CDO1174-caaacc13	Steudelli (CIho 2266)	Bjornstad et al. (2004)
QTLCB3H.3*	3H	448.4	61.5	11_0823	SBCC154 (Cebada)	Hofmann ***et al***. (2013)
QTLCB3H.4*	3H	449.9	37.8	11_0205	SBCC154 (Cebada)	Hofmann ***et al***. (2013)
QTL.3H-Shyri	3H	454.9	27.0	chr3H_498944660	Shyri	Zantinge et al. (2019)
QTLIA3H.5*	3H	455.3	4.9	YLM—MWG680	Abyssinian (CIho 668)	Grønnerød ***et al***. (2002)
QTLIA3H.2*	3H	455.3	6.8	YLM—MWG680	Abyssinian (CIho 668)	Grønnerød ***et al***. (2002)
QTLIA3H.4*	3H	455.3	13.8	YLM—MWG680	Abyssinian (CIho 668)	Grønnerød ***et al***. (2002)
QTLIA3H.1*	3H	455.3	16.5	YLM—MWG680	Abyssinian (CIho 668)	Grønnerød ***et al***. (2002)
QTLIA3H.6*	3H	455.3	18.3	YLM—MWG680	Abyssinian (CIho 668)	Grønnerød et al. (2002)
QTLIC3H.1*	3H	455.3	15.8	MWG680—agtc-17	CIho 11549 (Nigri Nudum)	Patil et al. (2003)
Rrs1 (BC240)	3H	455.3	-	cMWG680	CPI 109853 (*H. spontaneum*)	Genger et al. (2003a)
QTLCDPB3H.1*	3H	455.3	141.3	11_0315	SBCC145 (Cebada Del Pais)	Hofmann et al. (2013)
QTLIS3H.1*	3H	455.3	5.7	YLM—MWG680	Steudelli (CIho 2266)	Bjornstad et al. (2004)
Rrs1 (Rh)	3H	455.3	-	cMWG680	Triton	Graner et Tekauz (1996)
Rrs1 (Rh)	3H	455.3	-	-	Armelle	Looseley et al. (2020)
QTLTritonRrs3Hnatural	3H	455.3	6.5	cMWG680	Triton	Wagner et al. (2008)
Rrs1 (Rh4[2])	3H	455.3	-	-	Modoc (CIho 7566)	Dyck et Schaller (1961)
Rrs1 (Rh3)	3H	455.3	-	-	Turk (CIho 14400)	Dyck et Schaller (1961)

Rrs1 (Rh3)	3H	455.3	-	-	Atlas 46 (CIho 7323)	Goodwin et al. (1990)
QTLIA3H.3*	3H	480.5	7.2	HVM27—fa1666	Abyssinian (CIho 668)	Grønnerød et al. (2002)
QTLRrsq1	3H	486.3	4.1	GBM1163	Vada (CIho 11765)	Shtaya et al. (2006)
qC07_3	3H	487.4	90.6	GBM1094—STSagtc17	CIho 3515 (PI 58052)	Coulter ***et al***. (2019)
qC147_3	3H	487.4	74.5	GBM1094—Bmag0112	CIho 3515 (PI 58052)	Coulter ***et al***. (2019)
qC174_3	3H	487.4	6.0	GBM1094—GMS0116	CIho 3515 (PI 58052)	Coulter ***et al***. (2019)
QTLSR-3H-2015	3H	503.4	5.1	bPb-7872	Yerong	Coulter et al. (2019)
Rrs4	3H	523.0	15.9	HVM36b—HVM60	CIho 11549 (Nigri Nudum)	Patil et al. (2003)
qS271_3	3H	547.7	7.1	2_0023—1_0253	Morex (CIho 15773)	Coulter et al. (2019)
QTLSR3H.3*	3H	560.9	5.5	11_2138111_11172	Saffron	Looseley et al. (2014)
QTLCW3H.3*	3H	564.0	4.0	11_10515—11_20612	Cocktail	Looseley et al. (2012)
QTLCW3H.1*	3H	564.0	20.0	11_10515—11_20612	Cocktail	Looseley et al. (2012)
QTLCW3H.2*	3H	564.0	13.0	11_10515—11_20612	Cocktail	Looseley et al. (2012)
Rrs1 (Halcyon)	3H	573.5	28.2	Xwg178	Halcyon	Genger et al. (2003b)
QTLSHS2000*	3H	573.5	6.8	Bcd22	Halcyon	Read et al. (2003)
QTLSHN1999*	3H	573.5	8.0	Wg178	Halcyon	Read et al. (2003)
QTLSHS2000*	3H	573.5	11.0	P13/M57-3	Halcyon	Read et al. (2003)
QTLSHN2001*	3H	573.5	13.1	P13/M57-3	Halcyon	Read et al. (2003)
QTLSHG2001*	3H	573.5	28.3	P13/M57-3	Halcyon	Read et al. (2003)
Qyrn3	3H	573.5	6.7	denso—ABG004	Regatta	Jensen et al. (2002)
QTLMK3H*	3H	601.4	2.1	denso—ABG004	Keel	Cheong et al. (2006)
QRh.S42-3H.a	3H	606.2	-	HVM62	ISR42-8 (*H. spontaneum*)	von Korff et al. (2005)
QTLRS7a	3H	606.2	2.5	P71m291g—HVM62	WI2291	Sayed et al. (2004)

QTLRS7b	3H	606.2	2.5	P71m291g-HVM62	WI2291	Sayed et al. (2004)
QTLRS6	3H	606.2	2.6	P71m291g—HVM62	WI2291	Sayed et al. (2004)
QSc.VlWa.4H.1	4H	0.2	6.3	GBM1501	Vlamingh	Wang et al. (2014)
Rrs16 Hb2	4H	1.7	-	MWG634	A17/1 (*Hordeum bulbosum*)	Pickering et al. (2006)
Rrs16 Hb1	4H	1.7	-	MWG634	A17/1 (*Hordeum bulbosum*)	Pickering et al. (2006
QSc.VlWa.4H.2	4H	1.7	17.0	bPb-9304	Vlamingh	Wang et al. (2014)
QTLVB4H.1*	4H	1.7	25.3	bPb-9304	Vlamingh	Wallwork et al. (2014)
QTLVB4H.3*	4H	1.7	44.0	bPb-9304	Vlamingh	Wallwork et al. (2014)
QRh.S42-4H.a	4H	98.9	5.4	GMS89	Scarlett	von Korff et al. (2005)
QTLCDP4BH.3*	4H	413.4	10.6	11_1316	SBCC145 (Cebada Del Pais)	Hofmann et al. (2013)
QTLVB4H.2*	4H	453.3	38.7	bPb-14836	Vlamingh	Wallwork ***et al***. (2014)
QTLVB4H.4*	4H	453.3	62.5	bPb-14836	Vlamingh	Wallwork ***et al***. (2014)
QTLHT4H*	4H	576.3	3.6	ABG472—MWG655C	TR306	Spaner et al. (1998)
Qsc4H.1-Shyri	4H	584.0	3.0	chr4H_602628307	Shyri	Zantinge ***et al***. (2019)
Qsc4H.2-Shyri	4H	584.0	3.0	chr4H_602628310	Shyri	Zantinge ***et al***. (2019)
QTLRrsq2	5H	594.4	3.9	Ebmac0701	Vada (CIho 11765)	Shtaya et al. (2006)
Qyrn4	5H	603.2	2.6	mlo	Alexis	Jensen et al. (2002)
Qsc5H-Shyri	5H	0.9	3.0	chr5H_1148266	Shyri	Zantinge et al. (2019)
qSUK7_5	6H	414.2	7.2	1_1135—2_0265	Steptoe (CIho 15229)	Coulter et al. (2019)
QTLCW5H.1*	6H	555.7	3.5	11_21077—11_11497	Cocktail	Looseley et al. (2012)
QTLIB6H*	6H	Unknown	2.6	ctaaca29—ctgacg13	Abyssinian (CIho 668)	Grønnerød et al. (2002)
QTLID6H*	6H	Unknown	-	-	Igri	Backes et al. (1995)
QTLRrsq4	6H	Unknown	4.2	E35M55-216	L94 (CIho 11797)	Shtaya et al. (2006)
QTLPostRrs6H271	6H	Unknown	3.6	V171100	Post (CIho 15695)	Wagner et al. (2008)
Qyrn6	6H	4.9	2.5	O12-475—IF3-400	Alexis	Jensen et al. (2002)

QTLMK6H*	6H	4.9	1.8	Bmac316	Keel	Cheong et al. (2006)
QSc.VlWa.6H.1	6H	6.0	12.0	Scald-seedling	WABAR2147	Wang ***et al***. (2014)
QSc.VlWa.6H.2	6H	6.0	12.9	1_1166	WABAR2147	Wang ***et al***. (2014)
Qsc1.6H.1-Seebe	6H	6.1	5.0	chr6H_6146622	Seebe	Zantinge *et al*. (2019)
Qsc2.6H.3-Seebe	6H	7.7	12.0	chr6H_7681197	Seebe	Zantinge ***et al***. (2019)
Qsc2.6H.2-Seebe	6H	8.5	12.0	chr6H_8562850	Seebe	Zantinge ***et al***. (2019)
qSUK7_6	6H	9.8	34.8	2_0262—1_1479	Steptoe (CIho 15229)	Coulter et al. (2019)
Qsc2.6H.5-Seebe	6H	10.9	12.0	chr6H_11180130	Seebe	Zantinge et al. (2019)
qC07_6	6H	11.8	11.8	U35_24165—GBS0346	CIho 3515 (PI 58052)	Coulter ***et al***. (2019)
qC147_6	6H	11.8	15.6	U35_24165—GBS0346	CIho 3515 (PI 58052)	Coulter ***et al***. (2019)
Rrs18 (qC174_6)	6H	11.8	97.1	U35_24165—U35_40281	CIho 3515 (PI 58052)	Coulter ***et al***. (2019)
Qsc2.6H.6-Seebe	6H	11.9	12.0	chr6H_12318331	Seebe	Zantinge ***et al***. (2019)
Qsc2.6H.4-Seebe	6H	12.0	12.0	bPb-6311	Seebe	Zantinge ***et al***. (2019)
QTLCH6H.1*	6H	13.6	2.2	ABG654	Harrington	Cheong ***et al***. (2006)
QTLSO6H*	6H	14.1	15.6	Bmag500—mwg516	O'Connor	Cheong ***et al***. (2006)
QTLRrsq3	6H	21.3	5.3	GBM1270	Vada (CIho 11765)	Shtaya et al. (2006)
Rrs13	6H	25.6	-	Cxp3—MWG916	BC Line 30 (CPI 71283)	Abbott et al. (1995)
Rrs13	6H	25.6	-	Cxp3—MWG916	Tantangara	Read et al. (1998) Genger et al. (2003b)
qS271_6a	6H	26.3	16.3	2_0232—1_0023	Steptoe (CIho 15229)	Coulter et al. (2019)
QTLHT6H*	6H	28.4	4.7	MWG916—WG223	Harrington	Spaner et al. (1998)
qS271_6b	6H	244.5	5.9	1_0129—1_1475	Morex (CIho 15773)	Coulter et al. (2019)
QTLSR6H.1*	6H	260.1	3.6	11_11097—11_10455	Saffron	Looseley et al. (2014)
QTLTritonRrs6H271	6H	314.6	3.2	HVM14	Triton	Wagner et al. (2008)
Qsc3.6H.7-Seebe	6H	545.5	4.0	chr6H_553522031	Seebe	Zantinge et al. (2019)
QTLCH6H.2*	6H	549.2	2.6	ABC154	Chebec	Cheong et al. (2006)
QTLID7H*	7H	Unknown	-	-	Igri	Backes et al. (1995)

QTLIB7H*	7H	Unknown	3.7	cacacg15—ctaaca36	Ingrid	Grønnerød et al. (2002)
QTLVB7H.3*	7H	1.6	3.3	bPb-25565	Buloke	Wallwork ***et al***. (2014)
QTLVB7H.2	7H	1.6	17.7	bPb-25565	Buloke	Wallwork ***et al***. (2014)
QTLCH7H.1*	7H	2.0	3.4	ABG312	Harrington	Cheong et al. (2006)
QTLIS7H.2*	7H	4.4	2.9	MWG2018—Bmag0206	Steudelli (CIho 2266)	Bjornstad et al. (2004)
QTLIS7H.1*	7H	4.4	5.1	MWG2018—Bmag0206	Steudelli (CIho 2266)	Bjornstad et al. (2004)
Rrs2	7H	5.2	10.0	AFLP14—P1D23R	Steudelli (CIho 2266)	Hanemann et al. (2009)
Rrs2	7H	5.2	-	AFLP14—P1D23R	Atlas 46 (CIho 7323)	Goodwin et al. (1990)
Rrs2	7H	5.2	-	AFLP14—P1D23R	Digger	Hanemann et al. (2009)
Rrs2	7H	5.2	-	AFLP14—P1D23R	Osiris (CIho 1622)	Hanemann et al. (2009)
QTLVB7H.4*	7H	5.4	5.6	2_0710	Buloke	Wallwork ***et al***. (2014)
QTLVB7H.1*	7H	5.4	11.2	2_0710	Buloke	Wallwork ***et al***. (2014)
QTLTritonRrs7H271	7H	7.7	8.3	Bmag767	Triton	Wagner et al. (2008)
Rh2	7H	10.8	-	CDO545	Atlas (CIho 4118)	Schweizer ***et al***. (1995)
Rh2	7H	10.8	-	CDO545	Atlas (CIho 4118)	Schweizer ***et al***. (1995)
Rrs12	7H	16.0	-	Acp2 Bmag7	AB200	Abbott et al. (1992) Genger et al. (2003b)
QTLSR-7H-2017	7H	69.9	5.8	bPb-4541	Franklin	Zhang et al. (2019)
QTLVixenRrs7H271	7H	144.8	4.0	HVM51	Vixen	Wagner et al. (2008)
QTLCW7H.2*	7H	334.9	8.0	11_11098—11_10169	Cocktail	Looseley *et al*. (2012)
QTLCW7H.1*	7H	334.9	10.0	11_11098—11_10169	Cocktail	Looseley *et al*. (2012)
QTLCW7H.3*	7H	334.9	8.0	11_11098—11_10169	Cocktail	Looseley ***et al***. (2012)
qS271_7	7H	587.6	4.3	2_1448—1_0885	Steptoe (CIho 15229)	Coulter et al. (2019)
QTLCH7H.2	7H	625.6	3.4	Bmac156	Harrington	Cheong et al. (2006)
Rrs15	7H	626.3	-	hvm49	BC line 35 (CPI 77132)	Genger et al. (2005)

Annex II.1: Test of independence of adult plant reaction of FIGS and GCP barley subsets to scald evaluated under field conditions in Ethiopia (2017-2018), and in Morocco (2018) based on different grouping of the disease reaction classes.

Locations	Sets	Group of 2 classes	Group of 3 classes
		χ^2 (P-value)	χ^2 (P-value)
Guich -Morocco 2018	GCP	7.338 (< .01)	9.592 (< .01)
	FIGS_Scald		
MCH-Morocco 2018	GCP	6.782 (< .01)	9.923 (< .01)
	FIGS_Scald		
Holetta-nursery 2017	GCP	0.387 (P = .534)	1.347 (P = .510)
	FIGS_Scald		
Holetta-nursery 2018	GCP	0.695 (P = .405)	5.464 (P = .065)
	FIGS_Scald		
Holetta-quarantine 2018	GCP	0.591 (P = .442)	2.010 (P = .366)
	FIGS_Scald		

Group of 2-classes includes reaction types I+R+MR and MS+S+HS, group of three classes include I+R, MR+MS, and S+HS.

Annex II.2: χ^2 tests of goodness of fit of different groups of disease reaction classes to scald under field conditions in Morocco and Ethiopia using FIGS and GCP subsets of barley.

Locations	Group of 6 classes	Group of 2 classes	Group of 3 classes
	χ^2 (P-value)	χ^2 (P-value)	χ^2 (P-value)
Guich -Morocco 2018	18.646 (< .01)	9.444 (< .01)	53.610 (< .01)
MCH-Morocco 2018	18.853 (< .01)	9.350 (< .01)	13.255 (< .01)
Holetta-nursery 2017	8.642 (P = .124)	0.582 (P = .446)	2.080 (P = .353)
Holetta-nursery 2018	8.807 (P = .066)	1.028 (P = .311)	6.848 (P = .033)
Holetta-quarantine 2018	8.344 (P = .080)	0.856 (P = .355)	2.700 (P = .259)

Group of 6-classes includes reaction types I, R, MR, MS, S, HS, group of 2-classes include I+R+MR and MS+S+HS, group of three classes include I+R, MR+MS, and S+HS.

Annex II.3: **C**omparison of modeling performance between four machine learning algorithms for the combined field data of reaction to scald in all barley accessions tested.

Best model	**BCART**	**KNN**	**RF**	**SVM**
Sensitivity	0.88	0.91	0.89	1.00
Specificity	0.43	0.26	0.39	0.00
Precision	0.76	0.71	0.74	0.67
Accuracy	0.73	0.69	0.72	0.67
Kappa	0.34	0.19	0.30	0.00
Accuracy Lower	0.69	0.65	0.68	0.62
Accuracy Upper	0.77	0.73	0.76	0.71
Accuracy Null	0.67	0.67	0.67	0.67
Accuracy (P-Value)	0.00	0.15	0.01	0.52

BCART, Bagged Carts; KNN, K-nearest neighbors; RF, Random Forest; SVM, Support vector machine

Annex III.1: Summary of QTL associated with resistance against three isolates of *Rhynchosporium commune* in barley AM2017 panel at the seedling stage. **Annex III.2.** Summary of QTL associated with *Rhynchosporium commune* resistance in barley AM2017 panel at the adult plant stage.

QTL	Peak SNP	[a]Chr	[b]QTL interval (Mb)	# of MTAs in QTL interval	*P-value*	Marker R^2 (%)	Allele frequency	[c]Allele Effect	TASSEL and GAPIT models
***APR-MCH18**									
qSc.APR1	JHI-Hv50k-2016-267965	4	605.577	1	1.64E-04	4.76	134	C (-7.8)	MLM(K+PCA),MLM(K+Q),MMLM,BLINK
qSc.APR2	JHI-Hv50k-2016-440870	7	1.96-4.86	4	1.03E-04	4.91	55	A (-9.03)	MLM(K+PCA),MLM(K+Q),BLINK,FarmCPU
***APR-Guich18**									
qSc.APR3	JHI-Hv50k-2016-43566	1	484.55-485.81	4	3.94E-05	5.56	40	C (-1.8387)	MLM(K+PCA),MLM(K+Q),MMLM
qSc.APR4	JHI-Hv50k-2016-206686	3	578.71	1	2.09E-04	4.47	260	A (-1.4852)	MLM(K+PCA),MLM(K+Q),MMLM,FarmCPU,BLINK
qSc.APR5	JHI-Hv50k-2016-231680	4	19.77-19.78	2	7.86E-05	5.08	95	A (-1.30166)	MLM(K+PCA),MLM(K+Q),MMLM,BLINK
qSc.APR6	JHI-Hv50k-2016-265990	4	602.22	1	3.30E-05	5.63	81	A (-1.42184)	MLM(K+PCA),MLM(K+Q),MMLM,FarmCPU,BLINK
qSc.APR7	SCRI_RS_114729	5	37.99	1	5.31E-05	5.35	163.00	A (1.2627)	MLM(K+PCA),MLM(K+Q),MMLM
qSc.APR8	JHI-Hv50k-2016-469294	7	75.124	2	8.39E-05	5.06	279	C (-1.9035)	MLM(K+PCA),MLM(K+Q),MMLM
***APR-Rommani-18**									
qSc.APR9	JHI-Hv50k-2016-4445	1	4.89-5.05	1	2.68E-05	5.77	296	C (-2.2502)	MLM(K+PCA),MLM(K+Q),MMLM
qSc.APR10	JHI-Hv50k-2016-254564	4	539.411-540.66	2	1.10E-04	4.87	34	G (1.6833)	MLM(K+PCA),MLM(K+Q),MMLM,BLINK
qSc.APR11	SCRI_RS_179398	4	606.16-608.35	5	9.69E-06	6.42	221	A (-1.2878)	MLM(K+PCA),MLM(K+Q),MMLM,BLINK,FarmCPU
qSc.APR12	JHI-Hv50k-2016-433362	6	571.58	1	1.07E-04	4.94	119	A (-1.35513)	MLM(K+PCA),MLM(K+Q),MMLM

*Environment and cropping season; APR-MCH (Marchouch), APR-Guich (Guich), APR-Rommani (Rommani)

[a] Chromosome

[b] Positions on the barley pseudomolecules Morex v. 2.0 2019

[c] Allele effect contributed by the respective marker on a 0–9 scale at the adult plant stage. The negative allele effect decreases the diseases severity (resistance) and the positive allele effect increases the diseases severity (susceptibility). The QTL indicated in bold text were significant with Bonferroni correction

Annex III.2: QTL alignment and candidate genes identified for the seedling resistance against three isolates of *Rhynchosporium commune* in barley AM2017 panel.

QTL	Peak SNP	Chr	Position Morex V2 (Mbs)	Gene identifier	Homology	References
SRT-SC-511						
qSc.SRT1	SCRI_RS_155612	2	27.6563	HORVU.MOREX.r3.2HG0109310	Disease resistance protein (CC-NBS-LRR class) family	Hofmann et al. (2013), Looseley et al. (2018)
qSc.SRT2	JHI-Hv50k-2016-107864	2	582.6740	HORVU.MOREX.r3.2HG0184950	DNA-directed RNA polymerase subunit beta	
qSc.SRT3	SCRI_RS_226361	7	4.8364	HORVU7Hr1G002700	Serine/threonine-protein kinase	Bjornstad et al. (2004), Thauvin et al. (2022)
qSc.SRT4	JHI-Hv50k-2016-483776	7	390.1641	HORVU.MOREX.r3.7HG0700570	Protein ENHANCED DISEASE RESISTANCE 2-like	
qSc.SRT5	JHI-Hv50k-2016-508670	7	614.9417	HORVU7Hr1G113370	LRR-repeat protein At5g22700-like	Wang et al. (2014), Thauvin et al. (2022)
SRT-SC-1122						
qSc.SRT6	JHI-Hv50k-2016-180055	3	400.1422	HORVU.MOREX.r3.3HG0275610	Pentatricopeptide repeat-containing protein	
qSc.SRT7	JHI-Hv50k-2016-180771	3	408.1374	HORVU.MOREX.r3.3HG0276500	CTP synthase family protein	Looseley et al. (2014)
qSc.SRT8	JHI-Hv50k-2016-181208	3	414.3801	HORVU.MOREX.r3.3HG0277290	Plant regulator RWP-RK family protein	Coulter et al. (2019)
qSc.SRT9	JHI-Hv50k-2016-182481	3	431.5177	HORVU.MOREX.r3.3HG0279830	Cysteine/Histidine-rich C1 domain family protein	Looseley et al. (2014)
qSc.SRT10	JHI-Hv50k-2016-182733	3	438.9457	HORVU.MOREX.r3.3HG0280440	Beclin-1-like protein	Williams et al. (2001)
qSc.SRT11	JHI-Hv50k-2016-183825	3	451.3086	HORVU.MOREX.r3.3HG0282120	Leucine-rich repeat receptor-like protein kinase	Grønnerød et al. (2002), Patil et al. (2003), Hofmann et al. (2013), Bjornstad et al. (2004), Graner and Tekauz (1996), Looseley et al. (2020), Wagner et al. (2008), Dyck and Schaller (1961), Goodwin et al. (1990), Genger et al. (2003a)

qSc.SRT12	JHI-Hv50k-2016-434888	7	1.9762	HORVU.MOREX.r3.7HG0635050	LRR receptor-like serine/threonine-protein kinase EFR	Wallwork et al. (2014), Cheong et al. (2006), Bjornstad et al. (2004), Thauvin et al. (2022)
SRT-SC-S611						
qSc.SRT13	JHI-Hv50k-2016-71369	2	19.2131			Hofmann et al. (2013), von Korff et al. (2005), Daba et al. (2020)
qSc.SRT14	JHI-Hv50k-2016-443516	7	7.6668	HORVU.MOREX.r3.7HG0639110	Guanine nucleotide-binding protein alpha-2 subunit	Wagner et al. (2008), Loosely et al. (2018), Looseley et al. (2012)
qSc.SRT15	JHI-Hv50k-2016-490465	7	508.9174	HORVU.MOREX.r3.7HG0716940	L-ascorbate oxidase	Looseley et al. (2012)

Annex III.3: QTL alignment and candidate genes identified for the adult plant stage resistance against *Rhynchosporium commune* in barley AM2017 panel

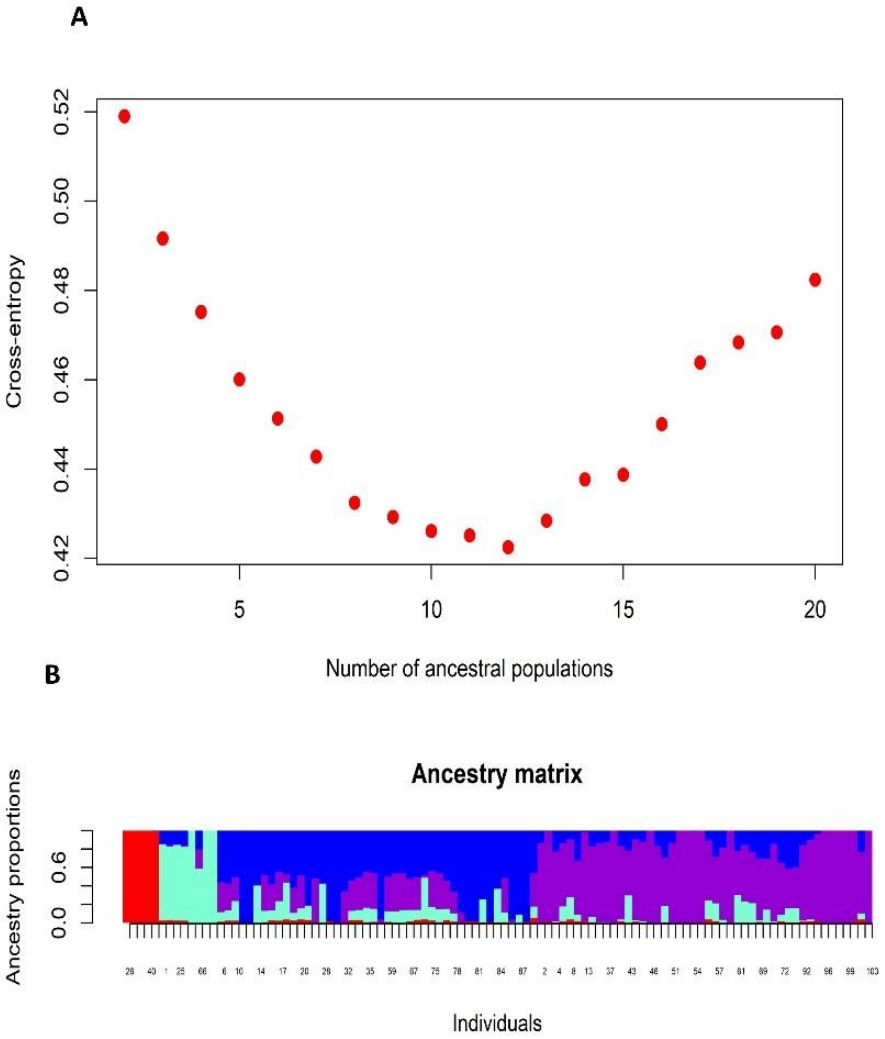
QTL	Peak SNP	Chr	Position Morex V2 (Mbs)	Gene identifier	Homology	References
APR-MCH18						
qSc.APR1	JHI-Hv50k-2016-267965	4	605.7778	HORVU4Hr1G083720	bZIP transcription factor 27	Jensen et al. (2002), Zantinge et al. (2019)
qSc.APR2	JHI-Hv50k-2016-440870	7	4.8325	HORVU.MOREX.r3.7HG0637270	Nuclease, putative	Hanemann et al. (2009), Goodwin et al. (1990), Daba et al. (2020), Thauvin et al. (2022), Bjornstad et al. (2004), Wallwork et al. (2014), Cheong et al. (2006)
	JHI-Hv50k-2016-439553	7	4.002	HORVU.MOREX.r3.7HG0636590	TSL-kinase interacting protein 1	
	JHI-Hv50k-2016-438638	7	3.376	HORVU7Hr1G001550	Very-long-chain 3-oxoacyl-CoA reductase 1	
	JHI-Hv50k-2016-438980	7	3.641	HORVU7Hr1G001750	NBS-LRR disease resistance-like protein	
APR-Guich18						
qSc.APR3	JHI-Hv50k-2016-43566	1	484.5590	HORVU.MOREX.r3.1HG0078000	Transmembrane protein 56-B	
	JHI-Hv50k-2016-43993	1	485.779	HORVU.MOREX.r3.1HG0078450	Ubiquitin carboxyl-terminal hydrolase 25	
	SCRI_RS_235724	1	485.809	HORVU.MOREX.r3.1HG0078480	FAR1-related sequence 5	

	JHI-Hv50k-2016-44088	1	485.810	HORVU.MOREX.r3.1HG0078480	FAR1-related sequence 5	
qSc.APR4	JHI-Hv50k-2016-206686	3	578.7114	HORVU.MOREX.r3.3HG0309560	Selenium-binding protein	Grønnerød et al. (2002), Loosely et al. (2014)
qSc.APR5	JHI-Hv50k-2016-231680	4	19.7783	HORVU.MOREX.r3.4HG0337450	Pentatricopeptide repeat-containing protein	
	JHI-Hv50k-2016-231677	4	19.78	HORVU.MOREX.r3.4HG0337450	Pentatricopeptide repeat-containing protein	
qSc.APR6	JHI-Hv50k-2016-265990	4	602.2248	HORVU.MOREX.r3.4HG0410190	Receptor-like protein kinase 4	Zantinge et al. (2019), Jensen et al. (2002)
qSc.APR7	SCRI_RS_114729	5	37.9994	HORVU.MOREX.r3.5HG0432280	Kinase interacting (KIP1-like) family protein	
qSc.APR8	JHI-Hv50k-2016-469294	7	75.1247	HORVU.MOREX.r3.7HG0663810	Aldose 1-epimerase, Fucosyltransferase	
	JHI-Hv50k-2016-469307	7	75.127	HORVU.MOREX.r3.7HG0663810	Aldose 1-epimerase, Fucosyltransferase	
APR-Rommani-18						
qSc.APR9	JHI-Hv50k-2016-4445	1	5.0419	HORVU.MOREX.r3.1HG0002370	Serine/threonine-protein kinase	Wang et al. (2014)
qSc.APR10	JHI-Hv50k-2016-254564	4	540.5062	HORVU.MOREX.r3.4HG0395710	Caleosin-related family protein	
	JHI-Hv50k-2016-254600	4	540.667			
qSc.APR11	SCRI_RS_179398	4	606.2172	HORVU.MOREX.r3.4HG0411620	Thyroid adenoma-associated protein homolog	Zantinge et al. (2019), Jensen et al. (2002), Looseley et al. (2012)
	JHI-Hv50k-2016-268095	4	606.168	HORVU.MOREX.r3.4HG0411600	Sugar transporter protein	
	JHI-Hv50k-2016-268154	4	606.21	HORVU.MOREX.r3.4HG0411620	Thyroid adenoma-associated protein homolog	
	JHI-Hv50k-2016-268158	4	606.212	HORVU.MOREX.r3.4HG0411620	Thyroid adenoma-associated protein homolog	
	JHI-Hv50k-2016-268616	4	608.352	HORVU.MOREX.r3.4HG0412330	Homeobox protein BEL1 homolog	
qSc.APR12	JHI-Hv50k-2016-433362	6	571.5888	HORVU.MOREX.r3.6HG0633580	Leucine-rich repeat domain superfamily	

Annex IV.1: AMOVA results for the studied barley genotypes based on geographic origin and on the subpopulation at k=4

Subpopulation at k=4					
	Degreeof freedom	Sumof square	Mean square Variance	Variance component	Percentageof variation
Between Subpop	4	315056.8	78764.2	1981.109	23.06132187
Between samples Within Subpop	98	1247898	12733.66	6124.152	71.2888914
Within samples	103	49991.2	485.3513	485.3513	5.649786733
Total	205	1612946	7868.031	8590.612	100
Geographic Origin					
Source of variation	Degreeof freedom	Sumof square	Meansquare Variance	Variance component	Percentageof variation
Between origin	3	122427.7	40809.25	618.6235	7.602889227
Between samples Within origin	99	1440527	14550.78	7032.715	86.43213867
Within samples	103	49991.18	485.3513	485.3513	5.964972102
Total	205	1612946	7868.031	8136.69	100

Annex IV.2: Values of the cross-entropy criterion for SNMF algorithm to evaluate several putative populations (K) ranging from K=2 to K=20 **(A)**. Population structure using sNMF fonction at K=4 **(B)**.



Annex IV.3: Summary of MTA associated with resistance against two isolates of *Rhynchosporium commune* in wild barley at the seedling stage.

MTA	SNP Marker	Chr	Position (bp)	Effect	P. value	Marker R^2 (%)
SRT-SC-1122**						
Msc.SRT1	SCRI_RS_127822	3	10762816	-2.27	4.39E-05	17.68
Msc.SRT2	JHI-Hv50k-2016-147336	2	765722555	-0.17	2.25E-04	15.27
Msc.SRT3	JHI-Hv50k-2016-134808	2	740414463	0.62	3.04E-04	14.56
Msc.SRT4	JHI-Hv50k-2016-147342	2	765723329	-2.68	4.84E-04	14.12
Msc.SRT5	JHI-Hv50k-2016-252629	4	542426620	0.75	5.30E-04	13.87
Msc.SRT6	JHI-Hv50k-2016-239839	4	99212403	-1.46	5.39E-04	10.94
Msc.SRT7	JHI-Hv50k-2016-239957	4	101099818	1.46	5.39E-04	10.94
Msc.SRT8	JHI-Hv50k-2016-240005	4	101723978	1.46	5.39E-04	10.94
Msc.SRT9	JHI-Hv50k-2016-256850	4	572901527	-0.03	5.45E-04	13.59
Msc.SRT10	BOPA2_12_31406	2	709519520	1.61	5.81E-04	13.72
Msc.SRT11	JHI-Hv50k-2016-216454	3	670445657	1.00	7.27E-04	10.66
Msc.SRT12	JHI-Hv50k-2016-379923	6	25291141	-2.14	7.33E-04	13.50
Msc.SRT13	JHI-Hv50k-2016-94431	2	480369815	1.81	7.38E-04	10.84
Msc.SRT14	JHI-Hv50k-2016-207271	3	644962781	0.92	8.02E-04	10.31
Msc.SRT15	JHI-Hv50k-2016-118894	2	703710471	-0.15	9.05E-04	13.21
Msc.SRT16	JHI-Hv50k-2016-54173	1	547772817	-2.25	9.09E-04	12.73
Msc.SRT17	JHI-Hv50k-2016-228197	4	5374043	-2.33	9.23E-04	12.95
Msc.SRT18	JHI-Hv50k-2016-472488	7	98424042	-1.70	9.59E-04	12.71
SRT-SC-511**						
Msc.SRT19	JHI-Hv50k-2016-115931	2	694692374	2.482	6.69E-06	18.69
Msc.SRT20	BOPA1_14832-296	2	704890469	1.439	3.54E-04	15.05
Msc.SRT22	JHI-Hv50k-2016-513099	7	642670653	-3.337	4.68E-04	14.56
Msc.SRT23	JHI-Hv50k-2016-159242	3	23631682	-1.331	6.01E-04	11.33
Msc.SRT24	JHI-Hv50k-2016-122729	2	712501396	1.862	6.09E-04	14.10
Msc.SRT25	JHI-Hv50k-2016-211117	3	658334577	1.478	0.00108	10.35

Annex IV.4: Summary of MTA associated with resistance at adult plant stage *commune* in wild barley

MTA	SNP Marker	Ch	Position (bp)	Effect	P. value	Marker R2 (%)
APR-Guich						
M.sc.APR1	JHI-Hv50k-2016-99992	2	589512031	2.34	2.99E-05	17.89
M.sc.APR2	JHI-Hv50k-2016-274292	4	641464419	-3.36	5.61E-05	17.45
M.sc.APR3	JHI-Hv50k-2016-108773	2	658629474	-4.50	1.19E-04	15.74
M.sc.APR4	JHI-Hv50k-2016-317701	5	553259198	-1.26	2.28E-04	14.71
M.sc.APR5	JHI-Hv50k-2016-499735	7	616337334	-2.58	2.84E-04	14.35
M.sc.APR6	JHI-Hv50k-2016-421716	6	555747524	3.75	3.67E-04	13.94
M.sc.APR7	JHI-Hv50k-2016-507218	7	632756816	-3.11	4.71E-04	14.17
M.sc.APR8	JHI-Hv50k-2016-250665	4	524744246	-2.28	6.46E-04	13.57
M.sc.APR9	JHI-Hv50k-2016-128643	2	725654187	1.63	7.79E-04	10.67
M.sc.APR10	JHI-Hv50k-2016-244102	4	458771700	-3.36	8.73E-04	12.57
M.sc.APR11	SCRI_RS_236521	2	759411949	-2.24	9.11E-04	10.14
M.sc.APR12	JHI-Hv50k-2016-451269	7	16899719	-3.10	9.30E-04	12.79
M.sc.APR13	JHI-Hv50k-2016-39445	1	504078214	1.50	9.90E-04	12.31
M.sc.APR14	JHI-Hv50k-2016-272747	4	640310734	-2.35	0.00102	12.68
APR-MCH						
M.sc.APR15	JHI-Hv50k-2016-458804	7	35194798	0.62	1.39E-05	19.26
M.sc.APR16	JHI-Hv50k-2016-194331	3	571305480	-5.36	4.62E-05	17.53
M.sc.APR17	JHI-Hv50k-2016-418384	6	545726882	-4.84	1.93E-04	12.43
M.sc.APR18	JHI-Hv50k-2016-276626	4	645760089	2.05	3.98E-04	11.30
M.sc.APR19	JHI-Hv50k-2016-382629	6	34583554	-2.15	4.66E-04	13.69
M.sc.APR20	JHI-Hv50k-2016-7989	1	7424883	-1.65	5.29E-04	10.85
M.sc.APR21	JHI-Hv50k-2016-513340	7	644417522	-1.71	6.23E-04	10.59

M.sc.APR22	JHI-Hv50k-2016-137311	2	745069584	-6.96	6.54E-04	13.13
M.sc.APR23	JHI-Hv50k-2016-395024	6	249836881	0.41	7.50E-04	12.90
M.sc.APR24	JHI-Hv50k-2016-306263	5	469496728	-2.40	7.72E-04	10.25
M.sc.APR25	JHI-Hv50k-2016-49355	1	533016564	-4.78	7.93E-04	12.81
M.sc.APR26	JHI-Hv50k-2016-95046	2	515283230	-4.61	8.78E-04	12.75
M.sc.APR27	BOPA2_12_30653	1	272697	2.17	9.54E-04	9.92
M.sc.APR28	JHI-Hv50k-2016-382628	6	34583535	-0.17	9.57E-04	12.50
M.sc.APR29	JHI-Hv50k-2016-382631	6	34583733	-0.17	9.57E-04	12.50
M.sc.APR30	JHI-Hv50k-2016-343321	5	617105185	-3.54	0.00102	12.39
M.sc.APR31	BOPA2_12_20335	2	503886204	-2.18	0.0011	12.26

Annex IV.5: Candidate genes identified for the seedling resistance against two isolates of *Rhynchosporium commune* in wild barley.

MTA	**SNP Marker**	**Chr**	**Position (bp)**	**Homology**	**Gene identifier**
***SRT-SC1122**					
Msc.SRT1	SCRI_RS_127822	3	10762816	Mannose-6-phosphate isomerase 2	HORVU.MOREX.r3.3HG0222320
Msc.SRT3	JHI-Hv50k-2016-134808	2	740414463	serine/threonine protein kinase	HORVU.MOREX.r3.2HG0208170
Msc.SRT4	JHI-Hv50k-2016-147342	2	765723329	Peptide transporter	HORVU.MOREX.r3.4HG0393720
Msc.SRT6	JHI-Hv50k-2016-239839	4	99212403	Autophagy-related protein 13	HORVU.MOREX.r3.4HG0349570
Msc.SRT7	JHI-Hv50k-2016-239957	4	101099818	Transcription factor VIP1	HORVU4Hr1G020540
Msc.SRT9	JHI-Hv50k-2016-256850	4	572901527	basic helix-loop-helix (bHLH) DNA-binding superfamily protein	HORVU.MOREX.r3.4HG0398470
Msc.SRT10	BOPA2_12_31406	2	709519520	Glycogen synthase 1	HORVU2Hr1G106410
Msc.SRT11	JHI-Hv50k-2016-216454	3	670445657	Glucan endo-1,3-beta-glucosidase GI	HORVU3Hr1G106140

Msc.SRT12	JHI-Hv50k-2016-379923	6	25291141	phospholipase D P1	HORVU.MOREX.r3.6HG0549950
Msc.SRT14	JHI-Hv50k-2016-207271	3	644962781	F-box family protein	HORVU.MOREX.r3.3HG0310270
Msc.SRT15	JHI-Hv50k-2016-118894	2	703710471	D-2-hydroxyglutarate dehydrogenase, mitochondrial	HORVU.MOREX.r3.2HG0195930
Msc.SRT16	JHI-Hv50k-2016-54173	1	547772817	Protein kinase superfamily protein	HORVU1Hr1G091230
Msc.SRT18	JHI-Hv50k-2016-472488	7	98424042	Progesterone 5 beta reductase	HORVU7Hr1G038590
SRT-SC511*					
Msc.SRT19	JHI-Hv50k-2016-115931	2	694692374	Growth-regulating factor	HORVU.MOREX.r3.2HG0193490
Msc.SRT20	BOPA1_14832-296	2	704890469	Glutamate receptor	HORVU.MOREX.r3.2HG0196290
Msc.SRT22	JHI-Hv50k-2016-513099	7	642670653	Vacuolar membrane protease	HORVU7Hr1G116080
Msc.SRT24	JHI-Hv50k-2016-122729	2	712501396	Phosphatidylinositol-4-phosphate 5-kinase family protein	HORVU.MOREX.r3.2HG0198920
Msc.SRT25	JHI-Hv50k-2016-211117	3	658334577	methyl esterase 1	HORVU3Hr1G098360

Annex IV.6: Candidate genes identified for the adult plant resistance against *Rhynchosporium commune* population in wild barley.

MTA	**SNP Marker**	**Chr**	**Position Mb**	**Homology**	**Gene identifier**
APR-Guich*					
M.sc.APR1	JHI-Hv50k-2016-99992	2	589512031	Calcium-binding protein 1	HORVU.MOREX.r3.2HG0174070
M.sc.APR2	JHI-Hv50k-2016-274292	4	641464419	DUF584	HORVU4Hr1G089030
M.sc.APR3	JHI-Hv50k-2016-108773	2	658629474	Ubiquitin carboxyl-terminal hydrolase family protein	HORVU2Hr1G093490
M.sc.APR4	JHI-Hv50k-2016-317701	5	553259198	Cilia- and flagella-associated protein 20	HORVU.MOREX.r3.5HG0495020
M.sc.APR5	JHI-Hv50k-2016-499735	7	616337334	DNA repair protein recA	HORVU.MOREX.r3.7HG0734260
M.sc.APR6	JHI-Hv50k-2016-421716	6	555747524	Clustered mitochondria protein	HORVU.MOREX.r3.6HG0623560
M.sc.APR7	JHI-Hv50k-2016-507218	7	632756816	D-3-phosphoglycerate dehydrogenase	HORVU.MOREX.r3.7HG0740480

M.sc.APR8	JHI-Hv50k-2016-250665	4	524744246	alpha/beta-Hydrolases superfamily protein	HORVU.MOREX.r3.4HG0391400
M.sc.APR9	JHI-Hv50k-2016-128643	2	725654187	jasmonate-zim-domain protein 12	HORVU2Hr1G112360
M.sc.APR10	JHI-Hv50k-2016-244102	4	458771700	F-box family protein	HORVU.MOREX.r3.4HG0383460
M.sc.APR11	SCRI_RS_236521	2	759411949	Tubby-like F-box protein	HORVU.MOREX.r3.2HG0215220
M.sc.APR12	JHI-Hv50k-2016-451269	7	16899719	Sulfite exporter TauE/SafE family protein, putative	HORVU.MOREX.r3.7HG0643880
M.sc.APR13	JHI-Hv50k-2016-39445	1	504078214	60S acidic ribosomal protein	HORVU.MOREX.r3.1HG0074540
M.sc.APR14	JHI-Hv50k-2016-272747	4	640310734	GATA transcription factor 2	HORVU4Hr1G088280
APR-MCH*					
M.sc.APR16	JHI-Hv50k-2016-194331	3	571305480	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein	HORVU.MOREX.r3.3HG0293920
M.sc.APR19	JHI-Hv50k-2016-418387	6	545727109	Histone acetyltransferase	HORVU.MOREX.r3.6HG0620230
M.sc.APR20	JHI-Hv50k-2016-276626	4	645760089	RING/U-box superfamily protein	HORVU.MOREX.r3.4HG0418370
M.sc.APR22	JHI-Hv50k-2016-7989	1	7424883	Caffeic acid 3-O-methyltransferase/L-tyrosine decarboxylase	HORVU1Hr1G003340
M.sc.APR23	JHI-Hv50k-2016-513340	7	644417522	F-box family protein	HORVU.MOREX.r3.7HG0746750
M.sc.APR24	JHI-Hv50k-2016-137311	2	745069584	heat-inducible transcription repressor (DUF639)	HORVU.MOREX.r3.2HG0209940
M.sc.APR25	JHI-Hv50k-2016-395024	6	249836881	tRNA pseudouridine synthase D	HORVU.MOREX.r3.6HG0583390
M.sc.APR26	JHI-Hv50k-2016-306255	5	469494134	Phospholipase D	HORVU.MOREX.r3.5HG0478570
M.sc.APR28	JHI-Hv50k-2016-49355	1	533016564	Oligopeptide transporter	HORVU.MOREX.r3.1HG0084740
M.sc.APR30	BOPA2_12_30653	1	272697	Endoribonuclease E-like protein	HORVU.MOREX.r3.1HG0000090
M.sc.APR33	JHI-Hv50k-2016-343321	5	617105185	MD-2-related lipid recognition domain-containing protein / ML domain-containing protein	HORVU.MOREX.r3.5HG0517120
M.sc.APR34	BOPA2_12_20335	2	503886204	ATP-dependent Clp protease ATP-binding subunit ClpB	HORVU.MOREX.r3.2HG0164120

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