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Intitulée

GENOME-WIDE ASSOCIATION STUDIES OF MULTIPLE TRAITS IN BARLEY (*Hordeum vulgare* L.)

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To all the hardworking and marginal farmers of the world…

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Abstract

Barley (*Hordeum vulgare* L.) is one of the most important cereals worldwide. It is grown mainly for animal feed and malt but it is also used for food in several regions across the globe. There is a constant need for breeding high yielding barley cultivars with improved resistance to biotic and abiotic stresses. Introgression of novel genes into elite lines is one of the main activities in a breeding program and lead to the development of superior varieties that can cope with the environmental challenges. The aim of this research is to investigate the phenotypic and genetic diversity of a world panel of barley accessions selected from ICARDA gene bank and breeding germplasm and to identify markers associated with some important traits in barley (agronomic, disease resistance) using the Genome-Wide Association Mapping (GWAS) approach. The Association Mapping panel (AM-2014) was genotyped using the 9K iSelect SNP markers and phenotyped for seedling and adult-plant resistance to net form and spot form of net blotch. Agro-morphological, yield and yield components traits were also scored. The phenotype and genotypic data was used in a mixed linear model analysis accounting for population structure and kinship matrix to detect significant marker-trait associations, corrected after using the False Discovery Rate (FDR) approach. Markers in high Linkage disequilibrium (LD) and within the 3.6 cM LD decay were considered as same QTL. In total, 140 QTLs were identified in the present study for the studied traits. Some genomic regions harbor QTL for more than one trait and, based on map comparisons, 58 QTL have been found to concur with previously mapped QTL. For all traits together, 82 novel QTL have been detected. Novel associations discovered in this study could be validated in different populations or through carefully generated bi-parental mapping populations and the QTLs mapped are valuable resources for marker-assisted selection (MAS) in barley.

Key words: Barley, GWAS, SNP, QTL, LD, net blotch, agronomic traits

Résumé

L'orge (*Hordeum vulgare* L.) est l'une des céréales les plus importantes au monde. Elle est cultivée principalement pour l'alimentation animale et le malt, mais elle est également utilisée pour la consommation humaine dans plusieurs régions du monde. Il existe un besoin constant d'amélioration génétique de variétés d'orge à rendement élevé, présentant une résistance améliorée aux stress biotiques et abiotiques. L'introgression de nouveaux gènes dans les lignées élites est l'une des principales activités d'un programme d'amélioration génétique visant le développement de variétés supérieures, capables de faire face aux défis environnementaux. Le but de cette thèse est d'étudier la diversité phénotypique et génétique d'une collection mondiale d'accessions d'orge, sélectionnées de la banque de gènes de l'ICARDA et d'identifier les QTL / marqueurs étroitement liés à certains caractères importants chez l'orge (traits agronomiques, résistance aux maladies) en utilisant l'approche de la cartographie d'association (GWAS). La collection d'orge (AM-2014) a été génotypée en utilisant les marqueurs moléculaires 9K SNP iSelect, et phénotypée pour la résistance des plantules et des plantes adultes a la forme nette et ronde de la rayure réticulée de l'orge. Les traits agro-morphologiques, le rendement et ses composantes ont également été évalués. Les données phénotypiques et génotypiques ont été utilisées dans l'analyse par le modèle linéaire mixte, tenant compte de la structure de la population et de la matrice de parenté pour détecter les associations marqueur-trait significatives, puis corrigées par l'approche FDR (False Discovery Rate). Les marqueurs en déséquilibre de liaison élevé (LD) et dans une désintégration de LD de 3,6 cM sont considérés comme le même QTL. Au total, 140 QTLs ont été identifiés dans la présente étude pour les caractères étudiés. Certaines régions génomiques abritent des QTLs pour plus d'un trait et, d'après les comparaisons cartographiques, 58 QTLs concordent avec les QTLs précédemment cartographiés. Pour tous les caractères confondus, 82 nouveaux QTLs ont été détectés. Les nouvelles associations découvertes dans cette étude pourraient être validées dans différentes populations ou à travers des populations de cartographie bi-parentale soigneusement générées, et les QTLs cartographiés constituent des ressources importantes pour la sélection assistée par marqueurs moléculaires (MAS) chez l'orge.

Mots clés : Orge, GWAS, SNP, QTL, LD, rayure réticulées, traits agronomiques

ملخص

يعتبر الشعير من أهم محاصيل الحبوب في جميع أنحاء العالم. ويزرع من أجل استعماله كعلف للحيوانات أو من أجل إنتاج شراب الشعير (الجعة)، هذا ويستخدم في التغذية في عدة مناطق في جميع أنحاء العالم. هناك حاجة مستمرة لتطوير أصناف من شعير مرتفعة الإنتاجية وتملك مقاومة للإجهادات الحيوية واللاحيوية .إن عملية نقل مورثات جديدة الى سلالات شعير مختارة هو أحد الأنشطة الرئيسية في أي برنامج تحسين وراثي، ويؤدي إلى تطوير أصناف متفوقة قادرة على مواجهة التحديات البيئية.

يهدف هذا البحث إلى دراسة التنوع الشكلي والوراثي لمجموعة مدخلات من سلالات الشعير من مختلف أنحاء العالم، والتي تم اختيارها من البنك الوراثي في المركز الدولي للأبحاث ايكاردا. وأيضاً تحديد المؤشرات المرتبطة بشكل وثيق ببعض الصفات الهامة للشعير (صفات زراعية، مقاومة الأمراض) باستخدام تقانة رسم خريطة الارتباط على كامل الجينوم GWAS. تم تحديد خريطة الارتباط (AM-2014) باستخدام الموديل 9K iSelect من تقانة تحديد الاختلاف على مستوى النكلوتيد الواحد (SNP)، ودرست الصفات الشكلية في مرحلة البادرات والنبات البالغ لمرض التبقع الشبكي للشعير، كما تم تسجيل الصفات الزراعية، الانتاجية والمكونات االمرتبطة بها. استخدمت البيانات الشكلية والوراثية في تحليل النموذج الخطي المختلط اعتمادا على بنية المجموعات ومصفوفة القرابة، وذلك لتحديد المؤشر المرتبط بشكل معنوي بالصفة وذلك بالاعتماد على معدل الخطأ (FDR). تم اعتبار المؤشرات ذات الاختلال التوازن الارتباطي (LD)عالي القيمة والتي تقع ضمن مسافة 3.6 سانتي مورغان (cM) تتبع لنفس الموقع الكمي للصفة (QTLs). في هذه الدراسة تم تحديد 140 موقع (QTLs) مرتبطة بالصفات المدروسة. بعض هذه المواقع ارتبطت بأكثر من صفة مدروسة، وبناءً على مقارنات الخرائط الجينية تم العثور على 58 موقع مورثي كمي مكتشفة في دراسات سابقة، هذا وتم اكتشاف 82 موقعًا مورثياً كمياً جديدًا (QTLs) بالنسبة لجميع الصفات المدروسة. هذا ويمكن التحقق من صحة الارتباطات الجديدة المكتشفة في هذه الدراسة، عبر تطبيقها على أصناف مختلفة أو من خلال إنشاء خريطة وراثية لعشيرة ثنائية الأبوية. إن المواقع التي تم تحديدها هي مورد قيّم لانتقاء مساعَد للعلامات (MAS) في الشعير.

الكلمات المفتاحية: الشعير، خريطة الارتباط على كامل الجينوم (GWAS)، الاختلاف على مستوى النكلوتيد الواحد (SNP)، موقع الصفة الكمي (QTL)، اختلال التوازن الارتباطي (LD)، مرض التبقع الشبكي، الصفات الزراعية.

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List of abbreviations

%	Percentage
AM	Association Mapping
APR	Adult Plant Resistance
AT	Allal Tazi
BAC	Bacterial Artificial Chromosome
BY	Biological (Biomass) Yield
cm	Centimetres
cM	Centi Morgan (genetic distance)
DArT	Diversity Array Technology
DH	Days to Heading
EST	Expressed Sequence Tagged
FDR	False Discovery Rate
GLM	General Linear Model
GWAS	Genome-Wide Association Studies
GY	Grain Yield
HW	Hectolitre (test) Weight
IAV-HII	Institut Agronomique et Vétérinaire Hassan II
IBSC	International Barley Sequencing Consortium
ICARDA	International Center for Agricultural Research in the Dry Areas
iSelect	Illumina SNP genotyping platform (7864 SNPs)
JS	Jemaa Shaim
LD	Linkage Disequilibrium
MAF	Minor Allele Frequency
MAS	Marker-Assisted Selection
Mbp	Mega base pair
MCH	Marchouch
MCMC	Markov Chain Monte Carlo
MLM	Mixed Linear Model
MMt	Million Metric tons
MTA	Marker-Trait Association
NDSU	North Dakota State University
NFNB	Net Form of Net Blotch
NJ	Neighbour Joining
PCA	Principal Component Analysis
PCoA	Principal Coordinate Analysis
PCR	Polymerase Chain Reaction
PH	Plant Height
PIC	Polymorphic Information Content
PM	Powdery Mildew
Ptm	*Pyrenophora teres* f. *maculata*
Ptt	*Pyrenophora teres* f. *teres*
QTL	Quantitative Trait Loci
REML	Restricted Maximum Likelihood

RFLP	Restriction Fragment Length Polymorphism
RIL	Recombinant Inbred Lines
SE	Sidi El Ayidi
SFNB	Spot Form of Net Blotch
SL	Spike Length
SNP	Single Nucleotide Polymorphism
SR	Seedling Resistance
SSR	Single Sequence Repeat
TKW	Thousand Kernel Weight
UPGMA	Unweighted Pair-Group Method using Arithmetic averages
USDA-ARS	United States Department of Agriculture - Agriculture Research Service

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Introduction

Barley is one of the most important cereals worldwide and nationwide. In Morocco for instance, it covers more than 2.0 million hectares, accounting for 30% of the total cereal production in the country (FAOSTAT, 2016). And unlike in industrialized countries where barley is mainly used for animal feed and malting, it is one of the staple food crops in many regions including but not limited to North Africa, East Africa and Central and South-west making barley an important socio-economic crop for smallholder farmers throughout the world. However, barley yield in many of these countries remains very low, due to poor management and inferior varieties. Thus, breeding superior high yielding varieties, resistant to biotic and abiotic constraints combined with the good agricultural practices and crop management is crucial in increasing productivity and improving livelihoods of smallholder farmers. With this main objective – of breeding superior varieties and provide useful information on barley genetics – in mind, the present study was elaborated in a framework collaboration between the *Institut Agronomique et Vétérinaire Hassan II* (IAV Hassan II) and the *International Center for Agricultural Research in the Dry Areas* (ICARDA) and consisted on the investigation of the diversity and QTL mapping of economically important traits in a world barley population.

Most of the modern genotypes cultivated today have descended from a small number of landraces and therefore, the genes controlling important traits have reduced diversity compared to the gene pool of landraces and wild relatives. In order to maintain plant genetic diversity and prevent the threats of constant genetic erosion, gene bank collections have been established and maintained. They constitute important reservoirs of natural genetic variations originating from a number of historical genetic events as a response to environmental stresses (Hoisington et al. 1999). Unlocking biodiversity held in gene banks and utilizing useful allele variations in breeding programs are essential for the crop improvement. However, many agriculturally important variations such as productivity-related traits, tolerance or resistance to biotic and abiotic stresses are controlled by several genes (polygenic) with major and minor effects, which complicate the breeding process. These complex traits are referred to as quantitative traits and the genomic regions containing genes or loci associated with a particular quantitative trait are known as Quantitative Trait Loci (QTL).

QTL detection and gene introgression in breeding lines have revolutionized plant breeding. In simple terms, QTL analysis is based on the principle of detecting an association between

phenotype and the molecular markers. Markers are used to partition the mapping population into different genotypic groups based on the presence or absence of a particular marker locus and to determine whether significant differences exist between groups with respect to the trait being measured (Young 1996; Tanksley 1997).

It is challenging to identify QTLs based on only traditional phenotypic evaluation. Identification of QTLs of complex agronomic traits and its utilization in crop improvement requires a genome-wide mapping of these QTLs using molecular markers. The identification of QTLs (DNA-linked markers) is useful for incorporating genes into improved cultivars via marker-assisted selection (MAS), map-based cloning of the tagged genes, and for a better understanding of the genetics of complex traits (Asins 2002). Linkage analysis and association mapping are the two most commonly used methods for QTL mapping.

Linkage mapping (bi-parental) involves the selection of two parental strains that have differences in the alleles that affect the variation in a given trait and development of a mapping population by crossing the selected parents. Phenotyping the mapping population for the trait of interest, genotyping the population with adequate number of uniformly spaced polymorphic markers and the construction of a genetic map lead to the identification of molecular markers (QTLs) linked to the trait. Although, usually leads to the identification of major genes/QTLs with large effects – contributed by one of the parents – it is costly, time consuming and the variations detected are limited to the selected parents for the mapping population development.

With the growing availability of genome sequence data and advances in technology for rapid identification and scoring of genetic markers, linkage disequilibrium (LD) based genome-wide association study (GWAS) has gained favour in higher plants, compared to traditional bi-parental mapping (Remington et al. 2001; Gupta et al. 2005; Mackay and Powell 2007; Cockram et al. 2008; Atwell et al. 2010). While conventional linkage analysis works on a population derived from a cross of parents divergent for a trait of interest, association mapping applies to collections of samples of a much wider germplasm base. GWAS offers an increased mapping resolution to polymorphisms at sequence level due to the intrinsic nature of exploiting historical recombination events and should therefore enhance the efficiency of gene discovery and facilitate marker assisted selection (MAS) in plant breeding (Gupta et al. 2005; Moose and Mumm 2008). Once the plant cultivars are genotyped with high-density markers, association

mapping is promising in resolving the genetic basis of complex traits of both economic and ecological importance.

With all this in mind, this study was designed to exploit the phenotypic and genetic diversity present in ICARDA barley gene bank and identify QTL responsible for variations in several important traits in barley as well as disease resistance to two important foliar diseases in barley. Overall, the main objectives of this study are: **1) To assess the genetic and phenotypic diversity, the extent and level of LD and the underlying population structure in a subset of barley genotypes; and 2) to map QTL associated with seedling and adult-plant resistance to net form and spot form of net blotch as well as five important agronomic traits in barley.**

I. State of art

1. Barley crop

Barley (*Hordeum vulgare L.*) is one of the most ancient crops among the cereals and has played a significant role in the development of agriculture (Ullrich, 2010). It is one of the major crop and a model species in the tribe Triticeae and the fourth major cereal crop after maize (*Zea mays*), wheat (*Triticum aestivum*) and rice (*Oryza sativa*) with a total production of 141.28 MMt (FAOSTAT, 2016). Barley has good adaptability and can be planted from the tropics to the marginal areas which led to its widespread cultivation and distribution throughout the world (von Bothmer et al. 1995) and on a global scale, it is known to be a drought, cold and salt tolerant crop and well adapted to low-input conditions.

Barley was a main staple crop since its domestication and was used as food throughout the old world. In the oldest Roman literature, barley was described as an award given to the champions of the games (Andrew 2008). The gladiators were called barley men or "hordearii" due to its rich dietary value. Barley with its good nutritional values is still used as a staple food in mountainous areas of Central Asia, in South-West Asia and Northern and Eastern Africa. It is believed that cultivated barley was domesticated around 10,000 years ago from its wild relative *Hordeum vulgare* ssp. *spontaneum* (C. Koch) in the Fertile Crescent (Bennett and Smith 1976; Badr et al. 2000; Salamini et al. 2002; Kilian et al. 2009). Many types of barley are grown throughout the world, due to its vast morphological and environmental adaptability and these can be: winter, spring, two-rowed, six-rowed, hulled, huskless, awned, awnless, hooded, malting, feed and food types. Presently, barley is used mainly as feed source for livestock (55-60%), for malting and distilling (30-40%) and to less extent for human consumption (3%) and 5% for seed (Ullrich, 2010). It the last 50 years, an average of 18 MMt of barley grain are traded annually, that accounts for about US$2.8 billion per year (FAOSTAT, 2013). The main exporters are France, Australia, Argentina, Germany and Ukraine and the main importers are Saudi Arabia, China, Belgium, Netherlands and Japan (FAOSTAT, 2013). Due to high yielding cultivars that ensure the continuous yield increase, the area under barley production is declining to less than 47 million hectares in 2016, compared to 78 million hectares in the 1980 (FAOSTAT, 2016). However, abiotic and biotic stresses are the main threats to barley

production and are the major focus areas in breeding programs to achieve further yield increase and yield stability.

Barley belongs to the genus *Hordeum* (Poaceae family and Triticeae tribe) which comprises of 32 species and around 45 taxa. All *Hordeum* species share the same basic morphology: a three single flowered spikelets at each rachis node called triplet, but a high level of genetic diversity is found among the species (von Bothmer et al. 1995; von Bothmer et al. 2003). Some *Hordeum* species are annual (*H. murinum* L. and *H. pusillum* Nutt) and some are perennials (*H. bulbosum* L. and *H. brevisubulatum* (Trin.) Link) and most are inbreeding but few species show self-incompatibility (eg. *H. bulbosum* L. and *H. brevisubulatum* (Trin.) Link) (von Bothmer et al. 2003).

Hordeum species can be grouped into three gene pools (Harlan and de Wet 1971). The primary gene pool includes cultivated barley and landraces along with *H. vulgare* ssp. *spontaneum*. Cultivated and wild barley are sexually compatible and some hybrid types can occasionally be found in the areas where they are growing alongside each other (Harlan 1971). The secondary gene pool comprises of *H. bulbosum* only, a very important species as it harbors many agronomically important and disease resistance genes that has been introgressed into the cultivated barley using doubled haploid production or embryo rescue techniques (Kasha and Kao 1970; Pickering and Johnston 2005; Sanei et al. 2011). The third gene pool comprise the rest of the *Hordeum* species, and are mostly polyploids with tetraploid and hexaploid genome types (Harlan 1971; von Bothmer et al. 1995; von Bothmer et al. 2003).

Ear-row number is an important trait in barley. In six-rowed barley all three spikelets at each rachis node are fertile and bear seeds whereas in two-rowed barley the lateral spikelets do not produce any seed (suppressed). The six-rowed spike phenotype is reported to be controlled by a major recessive allele *vrs1* (2H) (Lundqvist et al. 1997), whereas the dominant allele of *Vrs1* suppresses the lateral spikelets in six-rowed genotypes resulting in a two-rowed phenotype (Komatsuda et al. 2007). The dominant allele *Vrs1* was found to be widespread in wild barley and confirmed previous findings that two-rowed barley is the ancestral form and six-rowed evolved later from two-rowed barley. A study by Ramsey et al. (2011) identified and characterized another locus *Vrs5* (*Int-C*) on chromosome 4H, responsible in controlling male fertility and lateral spikelets in two-rowed barley in a 190 two-rowed and six-rowed barley cultivars.

Growth habit is another important classification used in barley and it is mainly under the control of vernalization genes at the *Vrn-H1, Vrn-H2,* and *Vrn-H3* loci (Takahashi and Yasuda 1971). The epistatic interaction between *Vrn-H1* and *Vrn-H2* determines winter and spring growth

habit (Szucs et al. 2007). Winter barley requires cold winter period before flowering. Most variation for winter alleles occur at *Vrn-H3* loci and is considered fixed genotypes but can provide adaptive variation in flowering behavior in barley (Cockram et al. 2007; Yan et al. 2006). Spring allele at *Vrn-H3* locus promotes early flowering and is found mostly in exotic germplasm (Takahashi and Yasuda 1971; Wang et al. 2010).

2. Genetics and genomics of barley

Cultivated barley is a self-pollinating diploid species with 2n=14 chromosomes and a large genome (~5500 Mbp) consisting of highly repetitive elements (Bennett and Smith 1976; Doležel et al. 1998; Wicker et al. 2008). It is a model plant due to its diploidy and shared genome collinearity with other Triticeae species, including the hexaploid wheat.

During the last three decades, a wide range of genomics-based technologies has been developed to facilitate the systematic analysis of the barley genome, to study the genetic basis of barley and isolate agronomically important genes. These resources include a large number of mapped molecular markers, Expressed Sequence Tags (EST) collections, Bacterial Artificial Chromosome (BAC) libraries, DNA arrays, mutant collections and large scale production of double haploids. Several genetic linkage maps were developed for barley using different markers (Haseneyer et al. 2010). The first molecular linkage map using Restriction Fragment Length Polymorphism (RFLP) markers was developed in 1991 (Graner et al. 1991; Heun et al. 1991b). Polymerase Chain Reaction (PCR) based genetic markers have revolutionized the development of genetic linkage maps and gene discovery.

These advances made in barley genomics led towards barley genome sequencing and the construction of a genome-wide barley physical map http://barleygenome.org (IBSC, 2012) that will largely contribute to the understanding of gene functions in the context of agronomically important traits (Table 1). Currently, high-throughput SNP genotyping platforms with a capacity to genotype several hundred genotypes with thousands of SNPs (9K and recently 50K) are available at low costs and have revolutionized the practical uses of genomics (IlluminaTM: Veracode, Goldengate and the iSelect chip assay, Affymetrix gene chip) (Close et al. 2004; Close et al. 2009; Atwell et al. 2010;). High resolution genetic maps together with the sequence information in barley has huge potential for candidate gene discovery.

Table 1: Barley genomics resources (reviewed by Sreenivasulu et al., 2008)

Database	**URL**	**Application**
Barley Genetic Stocks	http://ace.untamo.net/cgi-bin/ace/searches/basic	Provides information on the morphological & genetic background of barley mutants and contains information on 736 barley translocation and duplication lines.
US Barley Germplasm	http://barleyworld.org/northamericanbarley/germplasm.php	Contains information on barley recombinant chromosome substitution lines and North American barley
EBDB	http://pgrc-35.ipk-gatersleben.de/portal/page/portal/	The European Barley Database (EBDB) contains passport and evaluation data of 155,000 barley accessions including the international barley core collection.
HarvEST	http://harvest.ucr.edu/	Barley EST database containing unigene sequences and the oligo design of Barley1 Affymetrix array. It also includes a 1000 barley SNP loci genetic map showing syntenic information with rice
NCBI Barley genome view	https://www.ncbi.nlm.nih.gov/mapview	Provides an overview about the available genomic and genomic survey sequences (GSS) of barley.
IBSC	http://barleygenome.org/	Activities of the International Barley Genome Sequencing Consortium (IBSC).
Barley genome	http://phymap.ucdavis.edu:8080/barley	Barley physical mapping database and available BAC clones together with the accompanying ESTs.
Barley physical map	http://pgrc.ipk-gatersleben.de/kuenzel/barleymap.html	Barley translocation breakpoints integrated into the Igri/Franka-derived RFLP linkage map.
Barley genomics	http://barleygenomics.wsu.edu/	Contains information about barley molecular markers, genetic maps, BACs and mutants.
Barley DB	http://ukcrop.net/perl/ace/search/BarleyDB	Contains information about barley germplasm, molecular markers, genetic maps and BACs.
Gramene	http://www.gramene.org/	Provides an overview of comparative maps of cereals including available updated molecular markers and maps of barley
GrainGenes	http://wheat.pw.usda.gov/GG2/index.shtml	Triticeae database provides an overview about available maps, genetic markers, QTLs and gene expression data.
Barley dbEST SSRs	http://www.genome.clemson.edu/projects/barley/ssr.dbest.html	15,182 barley simple sequence repeats (SSR) were predicted using the available 328,724 dbEST dataset.
Barley SNP database	http://bioinf.scri.ac.uk/barley$_$snpdb	Barley SNP linkage map
Barley RFLP database	http://pgrc.ipk-gatersleben.de/rflp/rflp.html	Contains data of mapped barley RFLP-markers from IPK.
Barley DArT	http://www.triticarte.com/content/barley$_$diversity$_$analysis.html	High density consensus map of barley DArT markers linking to existing SSR, RFLP and STS loci.
BarleyBase	http://www.plexdb.org/plex.php?database=Barley	An online dataset for storing and visualizing gene expression data of the Barley 1 GeneChip Affymetrix array.

3. Genome-Wide Association Studies (GWAS)

Association mapping or genome-wide association studies (GWAS), also known as linkage disequilibrium (LD) mapping refers to the non-random association of alleles at different loci (Slatkin 2008). It is the statistical association of genotype or SNP haplotype in a population to their phenotype (Rafalski 2010). Genome-wide association studies are highly successful in human genetics (Syvanen 2005). Several complex disease loci were identified in human populations using GWAS approach (International HapMap Consortium 2007; The International Hapmap Consortium 2005; Wellcome Trust Case Control Consortium 2007). In plants, GWAS was reported initially on self-pollinated model plant *Arabidopsis thaliana.*

The extent of LD differs depending on crops and gene pools and the loci that are close to each other tend to have strong LD (Slatkin 2008). The extent of LD determines the number of markers required to cover the genome and the resolution marker-trait association (Myles et al. 2009). High LD in a population indicates that fewer markers are required to detect the marker-trait associations, but with low resolution, whereas low level LD indicates that dense marker coverage is needed but the mapping resolution is higher (Myles et al. 2009; Waugh et al. 2009). The genome-wide LD decay was found rapidly decaying within 50 kb region in *Arabidopsis* (Remington et al. 2001; Thornsberry et al. 2001; Nordborg et al. 2005). Further, Aranzana et al. (2005) have successfully identified flowering time and disease resistance related genes using GWAS approach in a population of 95 *A. thaliana* accessions.

In barley, the level of LD is mainly determined by the pedigree and selection pressure of the germplasm. The extent of LD is higher in cultivated barley whereas low LD is observed in landraces and wild populations (Flint-Garcia et al. 2003) and thus, it is crucial to have the required marker coverage (number and distribution of markers) for an effective whole-genome association scans. Further to the level of LD, GWAS depend on the underlying structure in populations under the study. Majority of barley association genetic studies reported structured populations based on ear-row number and growth habits (Wang et al. 2010; Waugh et al. 2010; Comadran et al. 2011; Pasam et al. 2012; Amezrou et al. 2017). Further, within the row type, population structure was observed in different studies due to geographical origin and breeding histories (Zhang et al. 2009; Rodriguez et al. 2012; Amezrou et al. 2018).

However, spurious associations can often occur due to the population structure. Population structure is a result of the complex relationship among genotypes and the non-random

distribution of genotypes within a population and thus allele frequencies are biased among the subpopulations. In order to correct for population structure, different statistical models are developed and implemented (Flint-Garcia et al. 2003; Mackay and Powell 2007) among them, general linear model (GLM) to control false positives in association studies (Devlin and Roeder 1999; Abecasis et al. 2000; Pritchard et al. 2000) and recently, a mixed linear model (MLM) that accounts for both population structure and relatedness (Yu et al. 2006). Determining the appropriate statistical models to avoid the detection of spurious associations in GWAS is an important pre-requisite (Stich et al. 2008) and in most cases, mixed models performs better and is extensively used in association studies (Atwell et al. 2010; Huang et al. 2010; Comadran et al. 2011).

Considering the limitations of bi-parental mapping to identify genes/QTL of interest, genome-wide association studies (GWAS), using a diverse germplasm to efficiently capture marker-trait associations is considered an alternative and more efficient approach to identify multiple loci (Flint-Garcia et al. 2003; Zhu et al. 2008; Myles et al. 2009). In comparison to bi-parental mapping, association mapping captures multiple allele segregation in natural populations and varietal historical data can be used directly to characterize phenotypes at genomic level (Kraakman et al. 2004; Cockram et al. 2010; Wang et al. 2011).

II. Objectives and methodology of the study

1. Objectives of the study

The main objectives of this study aim are:

1. To determine (i) the genetic and phenotypic diversity of ICARDA's barley germplasm adapted to low input conditions, (ii) the extent and level of LD and (iii) the underlying population structure in a barley population of cultivars, advanced breeding lines and landraces frequently used in ICARDA's barley breeding programs.

Unlike bi-parental mapping where a mapping population should be developed from a widely different parents in the mean of phenotypic value of the trait of interest, genome-wide association mapping can be performed in set of different, yet unrelated germplasm. However, it is crucial to have a diverse collection of germplasm in terms of the phenotype of interest to capture significant associations. On the other hand, LD has to be determined before running genome-wide association studies. The resolution of GWAS and the cut-off of QTLs is based on the LD decay. Lastly, it is important to account for the population structure and relatedness in the GWAS analysis to reduce the false positives. In overall, the main objective of this chapter is to demonstrate the suitability of the population used in this study in GWAS.

2. Genome-wide association for net form of net blotch (*Pyrenophora teres* f. sp. *teres*) seedling and adult-plant resistance.

Foliar diseases are among the yield reducing constraints on barley. Net form of net blotch (NFNB) caused by *Pyrenophora teres* f. sp. *teres* (Ptt) is considered one of the major diseases in most barley growing regions around the world. In order to identify new sources of quantitative resistance, genome-wide association scan was performed using two isolates of Ptt, under controlled conditions. In addition, net form of net blotch resistance under field conditions was also studied at the adult-plant stage. The results may provide a foundational information on mapping Ptt resistance and a more insight on the race specific and non-race specific resistance at genome-wide level in barley that can be used in barley breeding programs.

3. Genome-wide association for spot form of net blotch (*Pyrenophora teres* f sp. *maculata*) seedling and adult-plant resistance.

Spot form of net blotch disease (SFNB) caused by *Pyrenophora teres* f. sp. *maculata* (Ptm) is the second form of net blotch of barley. Compared to NFNB, it is an emergent disease in many barley-growing regions around the world and the yield losses due to SFNB have become increasingly severe in recent years. Similar to NFNB, the objective of this chapter is to identify new sources of resistance at the seedling (using one isolate under controlled conditions) and adult-plant stages in field conditions using genome-wide association scan. The results of this study may provide a foundational information on the race specific and non-race specific resistance at genome-wide level in barley that can be used in barley breeding programs through Marker-Assisted selection (MAS) or gene pyramiding.

4. Genome-wide associations for agronomic traits (morphological, yield and yield components).

One of the most important objectives in barley breeding is to increase yield per se and its stability, through the understanding of the genetic basis of complex agronomic and developmental traits, usually under the control of major and minor QTLs. In order to dissect these traits and identify responsible QTLs at molecular level, GWAS was performed for seven traits (developmental traits, yield and yield related components) across multi-environmental field trials. The results may provide important genetic information on the QTLs/genes conditioning these important traits and the significant marker haplotype analysis should provide a useful resource for marker-assisted and genomic selection in future.

2. Material and methods

2.1. Plant materials

As stressed earlier, it is crucial to have a highly diverse mapping population to capture highly significant marker-trait associations of the trait of interest. The International Center for Agricultural Research in the Dry Areas (ICARDA) has the global mandate for barley improvement among the Consultative Group for International Agricultural Research (CGIAR) centers and holds one of the largest barley accessions (more than 30,000 barley accessions including wild relatives, landraces, and cultivars) in its gene banks. To construct the

association mapping panel (AM-2014) used in this study, a total of 336 barley genotypes including advanced breeding lines, cultivars, and landraces from ICARDA and other sources were used, representing much diversity present in the ICARDA's spring barley gene pool adapted to different agro-ecological environments. Annex 1 provides all the available information on this collection.

The barley genotypes selected for this study represent resistance/tolerance or susceptibility to abiotic (drought and heat) and biotic (foliar diseases including net form and spot form of net blotch, powdery mildew) stresses. The genotypes were selected from the two main ICARDA spring barley breeding programs targeted towards: low-input (230 genotypes), designed for stressed conditions (moisture and soil fertility) and high-input (82 genotypes), targeting regions with favorable growing conditions. The remaining 24 genotypes are frequently used for both conditions.

Further, the genotypes represent the three barley end-use objectives, namely feed, food, and malt barley. While designing the association mapping panel, appropriate consideration was given to select representative genotypes from both two-row (137) and six-row barley (199). All accessions are of spring growth habit and the genotypes showing winter or facultative growth habit were removed from the collection. Furthermore, the panel can also be classified as hulled (276), primarily used for feed and malting purposes, and hulless (60) barley for food uses.

2.2. Genotyping

The Illumina iSelect SNP array platform using 9k chip was used to genotype DNA sampled from the association mapping panel. Single plants of each line were grown in a greenhouse and the leaf tissue was lyophilized. Genomic DNA was extracted using the method described in Slotta et al. (2008).The barley genotypes were genotyped based on Illumina's Inifinium Assay (Illumina, San Diego, CA, USA) at the Cereal Crop Research Unit, USDA-ARS, Fargo, ND, USA. Allele calls were made from the successful 7,842 SNP markers. Further, SNP markers with >10% missing data points and <5% minor allele frequency were excluded from further analysis. A total of 6519 SNPs that passed the quality control criteria were subsequently used for analysis of population structure, linkage disequilibrium and genome-wide association scans. Throughout the study, all the analyses relied on the marker positions which were published as the barley iSelect consensus map (Munoz-Amatriain et al. 2014).

2.3. Population structure and linkage disequilibrium

Analysis of the population structure among barley genotypes was performed using the Bayesian model based analysis implemented in the STRUCTURE v2.3.4 (Hubisz et al. 2009; Falush et al. 2003; Pritchard et al. 2000). Each individual is assigned to different groups according to a membership coefficient (Q_i; RQ_i = 1.0). The posterior probabilities were estimated using the Markov Chain Monte Carlo (MCMC) method. The number of hypothetical populations (K) tested was from 1 to 7. For each K, 5 runs were set and the MCMC chains were run with a 100,000 burn-in period, followed by 100,000 iterations using the admixture model with correlated allele frequencies. The most likely number of sub populations was determined using the ΔK (Evanno et al. 2005) implemented in Structure Harvester (Earl and vonHoldt 2012). Furthermore, the genetic structure of the collection was also analysed by using Principal Coordinate Analysis (PCoA).

The estimates of the linkage disequilibrium (LD) of SNPs were determined for pairs of loci using the software package Tassel 3.0 (Bradbury et al. 2007). The squared allele frequency correlations (r^2) (Weir 1979) was calculated for each intra chromosomal combination between mapped SNPs. The distribution and extent of LD were visualized by plotting intra-chromosomal r^2 values against the genetic distance in cM for all inter-chromosomal marker pairs using nonlinear regression as described in Remington et al. (2001) and implemented in SAS 9.3.

2.4. Field experiments

In order to generate robust phenotypic data, multi-environmental field trials were performed. Evaluations of agronomic traits and screening of disease resistances were carried out at five Research Stations in Morocco. The experiments were carried out in Alpha-lattice design for two seasons (2014-15 and 2015-16) with two replications in Marchouch (MCH), Jemaa Shaim (JS), Sidi El Ayidi (SE), Sidi Allal Tazi (AT) and the experimental station of IAV Hassan II (IAV-HII), Rabat.

Seeds (10g) were sown in the two replicates for each genotype in paired rows of 1m with 30cm spacing between the rows and 1m space between the blocks. The experiments are conducted using the recommended agronomic packages for sowing rate and other inputs. Table 2 gives an overview on the research stations and Table 3 on the traits recorded in each environment.

Table 2: Research stations used for field experiments in Morocco

Research station	Code	Climate	Latitude	Longitude	Altitude (m)
Marchouch	MCH	Favourable	33.61 N	6.7 W	213
Sidi Allal Tazi	AT	Favourable	34.52 N	6.3 W	13
Sidi El Ayidi	SE	Intermediate	33.12 N	7.6 W	321
Jemaa Shaim	JS	Semi-Arid	32.40 N	8.7 W	170
IAV Hassan II	IAV	Favourable	33.97 N	6.8 W	10

Table 3: Agronomic and disease reaction traits evaluated during 2014-15 and 2015-16 seasons in seven environments in Morocco

Trait/Environment	MCH.15	JS.15	SE.15	IAV.15	SE.16	IAV.16	AT.16
Days to heading (DH)	x	x			x		x
Days to maturity (DM)	x						
Plant height (PH)	x	x			x		x
Spike length (SL)	x	x			x		x
Grain per spike (G/S)	x	x			x		
Biomass (BY)	x	x			x		x
Grain yield (GY)	x	x			x		x
Harvest Index (HI)	x	x			x		x
Thousand kernel weight (TKW)	x	x			x		x
Net for of net blotch (NFNB)	x	x	x	x	x	x	x
Spot form of net blotch (SFNB)	x	x	x	x	x	x	x
Powdery mildew (PM)	x	x					
Hectoliter (test) weight (HW)	x	x			x		x

2.5. Association mapping analysis

GWAS was conducted for each trait separately (agronomic traits and disease resistance) using Tassel 5.0 software package (Bradbury et al. 2007). Before analyses, phenotypic data were transformed to a more normal distribution with the Box-cox transformation method (Box and Cox 1964). The mean genotype reaction from experiments was used as the response factor in the analysis. The mixed linear model (MLM), accounting for the population structure (Q) and relative kinship (K) matrix as covariates (MLM: Q+K), was used to identify SNP markers

associated with different traits. The model equation as implemented in TASSEL software is expressed as (Yu et al. 2006):

$$\mathbf{y} = \boldsymbol{X}\boldsymbol{\beta} + \boldsymbol{S}\boldsymbol{\alpha} + \boldsymbol{Q}\mathbf{v} + \boldsymbol{Z}\mathbf{u} + \mathbf{e}$$

Where **y** is a vector of the phenotypic observation; **β** is a vector of fixed effects other than SNP or population group effects; α is a vector of SNP effects (QTL); **v** is a vector of population effects; **u** is a vector of polygene background effects; e is a vector of residual effects; ***Q*** is the population structure matrix relating **y** to **v**. ***X, S*** and ***Z*** are incidence matrices relating **β, α** and **u**, respectively (Yu et al. 2006). This model was demonstrated to result in best approximation to the expected cumulative distribution of *P* values and showed a good fit by decreasing the amount of spurious associations compared with the other models (Yu et al. 2006). A threshold of $P < 0.001$ was applied to identify significant marker-trait associations and for each significant marker, the positive false discovery rate (pFDR at $q<0.05$) was estimated to avoid false positive associations (Benjamin and Hochberg 1995). Phenotypic variation (R^2) and the marker effect was computed in Tassel 5.0 statistical output for each significant marker. Genetic positions of unknown significant markers were anchored to a POPSEQ position (Mascher et al. 2013) and further, a region is defined as a single QTL if the adjacent co-segregating significant markers are in less than the LD decay (genetic distance) with significant and strong local LD (with $r^2 > 0.15$). The QTL nomenclature for identified disease resistance QTL was done according to Grewal et al. (2008) and the POPSEQ position of significant markers was added to distinguish different QTL on the same chromosome (e.g. *QPtts-6H-54*). The growth stage is not mentioned in case a given QTL is conferring resistance at both growth stages. The QTL nomenclature for the agronomic traits was done by putting the phenotypic trait abbreviation (e.g. GY for grain yield) followed by the chromosome number and the position of significant markers (e.g. *GY-1H-19*).

III. Chapter 1: Genetic and phenotypic diversity of AM-2014 panel

Abstract

Plant breeders are interested in using diverse genotypes in hybridization that can segregate for traits of importance with possible genetic gain. Information on molecular and agro-morphological diversity helps breeder in reducing the effort for parental selection and this information helps advancement of generations. Phenotypic and molecular diversity study using 24 traits (agronomic and disease) and 6519 SNPs in a diverse collection of 336 spring barley genotypes was carried out at Marchouch and Jemaa Shaam research stations in Morocco. Based on structure and multivariate analyses, strong differentiation between the two-and six-row types was observed. The linkage disequilibrium (LD) decay of current collection (for the combined population) was up to 3.58 cM (r^2=0.15) while LD decay were estimated to 3.91 and 2.36 cM for two-and six-row barley, respectively. PCA of agronomic and disease reaction traits revealed that grains per spike, net form of net blotch (NFNB), spot form of net blotch (SFNB), and 1000-kernel weight (TKW) were the most discriminatory traits in the current collection. Association mapping in the two independent populations (two-row and six-row) will be ideal for identification of markers and QTL related to traits. The generated information on relatedness between individuals will help identify diverse genotypes for breeding programs.

Introduction

Assessment of the extent and nature of genetic variation in crop species has important implications in breeding, plant improvement, and conservation of plant genetic resources. The fact that barley is able to grow in different environments is due to its shaped diversity, accumulating a rich pool of genes as a result of adaptation to wide environments and survival under harsh conditions (Grando et al. 2001). Genetic diversity studies are important tools that help crop improvement by identification of diverse parental lines for hybridization and to introgress desirable genes into elite germplasm (Chakravorty et al. 2013; Gyawali et al. 2013). An extensive amount of data has been generated from genetic and phenotypic diversity surveys in wild and cultivated barley (Munoz-Amatriain et al. 2014; Comadran et al. 2009; Orabi et al. 2007; Brantestam et al. 2006; Feng et al. 2006; Malysheva-Otto et al. 2006; Pandey et al. 2006;

Chabane et al. 2005; Hou et al. 2005; Hamza et al. 2004; Baek et al. 2003; Matus and Hayes 2002; Struss and Plieske 1998; William et al. 1997). These studies also provide information about resource allocation that affect the long-term maintenance of diverse germplasm collections (McClean et al. 2012). An understanding of diversity and genetic structure is also important for association mapping since population structure can lead to spurious associations and a control can be used to reduce false positives. High-throughput genotyping platforms and candidate gene studies have promoted association mapping as a viable approach for quantitative trait locus (QTL) mapping as an alternative to the traditional QTL mapping, by using the recombination events from multiple lineages and to exploit the natural variation in large samples. Genotyping a diverse collection will help identify genomic regions of interest that control phenotypic variation. The barley 9K iSelect Illumina SNP platform gives whole genome coverage and an adequate genetic characterization of germplasm collections, which will make the diversity contained in a given collection efficiently accessible to barley breeders (Munoz-Amatriain et al. 2014; Comadran et al. 2009).

However, the success of association mapping depends on the extent and patterns of linkage disequilibrium (LD). The extent of LD in a given population determines the density of markers required for whole genome scan that have implications for identification of candidate genes associated with traits of interest (Szalma et al. 2005). Patterns of LD help understand the regions of low LD that has implications for breeder's selection. The overall LD helps understand the population genetic processes involved for shaping the present diversity in plants (Iqbal et al. 2012; Gurung et al. 2011; Malysheva-Otto et al. 2006; Gupta et al. 2005; Flint-Garcia et al. 2003). Basically, LD is affected by mating systems, recombination, selection, and genetic bottlenecks (Hamblin et al. 2011; Flint-Garcia et al. 2003). It is then important to know the population structure and the diversity of the population that can be used for association mapping. The objectives of this chapter is to explore genetic and phenotypic diversity of the collection and to determine the patterns of population structure and LD as well as the marker coverage and its suitability for genome-wide association mapping studies.

1. Phenotyping

Evaluations of agronomic traits and screening of disease resistances were carried out at two research stations in Morocco. The experiments were carried out in alpha-lattice design with two replications during 2014-15 season in Marchouch (MCH), and Jemaa Shaim (JS) research stations. Data was recorded at both stations for agro-morphological and yield components,

including days to heading (DH), days to maturity (DM), plant height (PH), spike length (SL), grains per spike (G/S), biological yield ha^{-1} (BY) grain yield ha^{-1} (GY), harvest index (HI=GY/BY), 1000-kernel weight (TKW) and hectoliter (test) weight in kg/hectoliter (HW). The genotypes were also screened for adult plant resistance (APR) to spot form of net blotch (SFNB), net form of net blotch (NFNB), and powdery mildew (PM) under natural infections. At adult stage (Zadoks GS 77-87), disease rating was visually recorded using double digit scale (00-99) where the first digit indicates vertical disease progress on the plant and the second digit refers to severity measured in the infected leaf area (Saari and Prescott 1975). Because of the drought conditions in Jemaa Shaim, PM resistance was evaluated at Zadoks growth stage 19-29 (pre-flowering) using 1-5 scale (1 as most resistant and 5 as most susceptible).

2. Phenotypic data analysis

Statistical analyses for all traits, in each location (MCH or JS), were done using Genstat v18 (VSN international, GenStat.co.uk). Multivariate analysis was performed on the measured qualitative and quantitative traits by using the principal component analysis (PCA) implemented in Genstat v18. In addition, ANOVA was performed to evaluate the environment and Genotype x Environment (G x E) effects, each trait was investigated to determine relatedness using Pearson's correlation coefficients. For multivariate analysis of agronomic traits, data from only 326 genotypes were considered that met the criteria of no missing data. The remaining 10 genotypes had at least one trait data missing and were excluded from PCA. For further investigation, a dendrogram based on mean traits from both locations was generated using hierarchical cluster analysis with group average linkage method in Genstat v18.

3. Genetic diversity analysis

Diversity statistics including genetic diversity, major allele frequency and Polymorphic Information Content (PIC) were analyzed using PowerMarker v3.25 software (Liu and Muse 2005). The phylogenetic analysis was conducted using Nei distance matrix (Nei 1972), computed by PowerMarker and used as input to generate the Unweighted Pair-Group Method using Arithmetic averages (UPGMA) dendrogram, viewed in TreeView X v0.5 (Page 1996). The genetic distance (D) among the genotypes was estimated by Unbiased Measures of genetic distance (Nei 1972). Genetic relationships among genotypes were further investigated by

principal coordinate analysis (PCoA) based on Nei genetic distance matrix in NTSYSpc 2.02i (Rohlf 2000).

4. Results

4.1. Phenotypic diversity

The phenotypic statistics like minimum, maximum, mean, standard error of the mean, and range of 24 traits are presented in Table 3. Using data for the quantitative and qualitative traits from both locations, the first three principal components (PCs) accounted for 66.4% of the total variability. The first PC explained 25.45% of the total variation (Fig 1). Particularly, G/S, NFNB and SFNB resistance in both locations were the variables with high positive loadings, while TKW had the largest negative loading. The second PC explained 21.79% of the total variation. In second PC, SFNB resistance in JS and TKW had the highest positive loadings while G/S and NFNB resistance in JS were the variables with the largest negative loadings. The third component that explained 19.16% of the total variation was associated with high positive loadings of NFNB resistance in both locations and TKW while the largest negative loading was associated with SFNB resistance in JS (Table 3). The ANOVA of agronomic traits are presented in Supplemental Tables 1a and 1b. Highly significant (P<0.01) effect of genotypes (G) was found for DH, PH, SL, NFNB, SFNB, G/S, BY, GY and HI (Sup table 1a) Significant (P<0.05) effect of environments (MCH and JS) was found for DH, PH, SL, NFNB, GY, BY, and HI index. Likewise, highly significant (P<0.01) effect of G×E interaction was observed for DH, SL, NFNB, SFNB, and GY. Significant (P<0.01) effect of genotypes was found for DM, TKW, HI, and PM-Adult in MCH while PM-Seedling was non-significant (P<0.05) in JS (Sup table 1a, 1b).

Table 4: Descriptive statistics and Principal Component Analysis (PCA) of phenotypic traits

Trait	Mean±SEM	Max	Min	Range	PC1	PC2	PC3
DH-MCH	103.3±0.30	130	90.5	39.5	0.004	0.03	0.001
DH-JS	115.7±0.38	147	89	58	0.0007	-0.02	-0.03
DM- MCH	154.2±029	172	104.5	67.5	0.013	0.015	0.002
PH- MCH	100.2±0.53	123.8	63.58	60.17	0.065	-0.05	-0.003
PH-JS	67.18±0.40	85	46.5	38.5	0.034	-0.006	-0.013
SL- MCH	7.717±0.08	12.22	4.867	7.35	-0.01	0.01	0.006
SL-JS	7.128±0.07	16.88	4.625	12.25	-0.006	0.005	0.003
G/S- MCH	48.66±1.04	83	23.33	59.67	[a]**0.246**	**-0.399**	-0.09
G/S-JS	48.52±0.80	90	22.5	67.5	**0.146**	**-0.231**	-0.06
BY- MCH	9.567±0.09	14.84	4.444	10.4	0.001	0.019	0.006
BY-JS	9.117±0.08	12.92	5.583	7.333	0.0006	0.003	0.003
GY- MCH	4.026±0.05	6.567	1.522	5.044	-0.002	0.01	0.001
GY-JS	2.873±0.03	4.458	1.048	3.41	-0.006	0.002	0.0006
HI- MCH	0.425±0.003	0.668	0.242	0.425	-0.0007	0.0001	-0.0001
HI-JS	0.32±0.002	0.59	0.1	0.49	0.002	0.01	0.002
TKW- MCH	45.32±0.28	60	27.5	32.5	**-0.118**	**0.802**	**0.181**
NFNB- MCH	66.51±1.71	93	00	93	**0.258**	0.068	**0.561**
SFNB- MCH	58.75±2.05	97	00	97	**0.219**	0.092	0.007
PM- MCH	76.26±1.43	50	00	3	0.001	-0.002	0.001
NFNB-JS	68.24±1.85	95	00	95	**0.426**	**-0.13**	**0.643**
SFNB-JS	71.23±1.62	95	00	95	**0.774**	**0.323**	**-0.413**
PM-JS	1.375±0.04	4	10	3	-0.001	-0.003	0.001
HW- MCH	68.33±0.34	83	56.5	26.5	-0.043	0.028	0.011

[a]Highlighted in bold are relevant characteristics that explained respective components

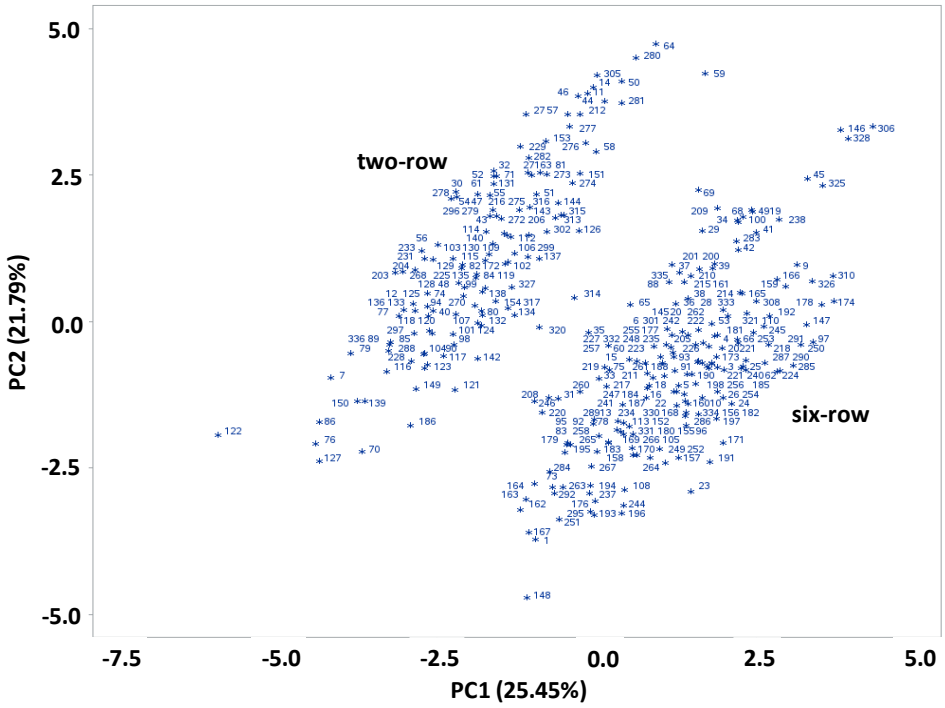

Figure 1: PCA based on genetic distance computed for 326 individuals using 24 agronomic traits at Marchouch (MCH) and Jemaa Shaim (JS)

4.2. Correlation between phenotypic traits

Correlation coefficients (r^2) were highly significant (P<0.001) in 46 out of the 276 trait combinations, where r^2 ranged from 0.01 to 0.96 (Fig. 2). The correlation coefficient and P-values are presented in a correlation matrix in Supplemental Table S2. High positive correlations ($r^2 \geq 0.5$) were found between BY and GY at both locations (BY-JS and GY-JS; BY-MCH and GY-MCH); DH-MCH and DM-MCH; G/S-JS and G/S-MCH with row type. High biomass imply high grain yield and similarly for days to heading and days to maturity. Highly significant negative correlations were found for G/S with, TKW and HW at both locations; HI-MCH with PH-MCH; and also between row type, SL, TKW and HW. The row type is playing a key role on the number of grain per spike, TKW and HW where the two-row types have less grains comparing to the six-row types and tend to have heavier and larger grains which determine the TKW and HW. Significant positive correlations between NFNB resistance at both locations was observed (r^2=0.39). Similarly, correlation was positively significant for resistance to SFNB at two locations (r^2=0.33). This indicates that the resistance or susceptibility was mainly governed by genetic factors and environment has very little effect on it.

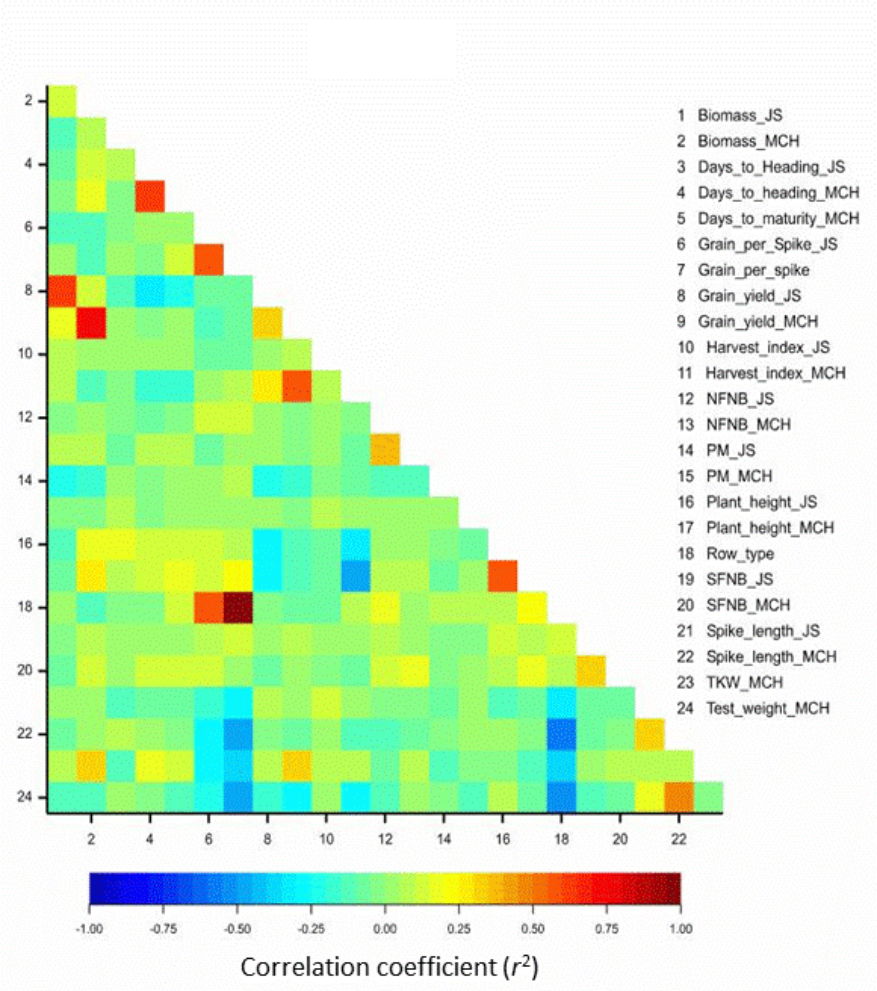

Figure 2: Pearson's correlations (r^2) among the 24 phenotypic traits measured for 326 barley genotypes

4.3. Cluster analysis of phenotypic traits

Hierarchical cluster analysis clearly classified barley genotypes into two main groups (Fig. 3a and 3c). Without exceptions, the two clusters separated two-row from six-row barley genotypes. Within a given cluster, genotypes aggregated into small groups based on their disease resistance or susceptibility, agronomic performance and morphological traits. Net blotch resistance, earliness, biomass, yield and plant height were the main traits of discrimination within the six-row and two-row clusters (Table 4, Fig. 3).

The lowest similarity (71.3%) was found between the genotypes AM-1 (Alanda/5/Aths/4/Pro/TolI//Cer*2/TolI/3/5106/6/Baca'S'/3/AC253//CI08887/CI05761) and AM-304 (CI3576) while the highest similarity (99.7%) was observed between the genotype AM-156 (Aths/Lignee686/4/Avt/Attiki//Aths/3/Giza121/Pue) and AM-18 (Massine/Arig8).

4.4. Genetic diversity and cluster analysis

A set of 6940 genome-wide SNPs was used to assess genetic diversity in the collection. After filtering for minor allele frequencies (MAF ≤ 0.05) and missing data (≥10%), a final subset of 6519 SNPs were used for further analyses (Table 5). Gene diversity and polymorphism information content (PIC) values on different chromosomes varied from 0.005 to 0.500 and 0.006 to 0.375, with average values of 0.366 and 0.290, respectively (Table 5).

Table 5: Marker information and diversity statistics of markers mapped in individual chromosomes

Chr	No. of SNP used[a] (after filtering)	Average distance per SNP (cM)	Major Allele Frequency	Gene Diversity	[b]PIC
1H	475	0.292	0.722	0.369	0.293
2H	804	0.207	0.742	0.346	0.277
3H	713	0.215	0.712	0.370	0.292
4H	472	0.258	0.706	0.381	0.300
5H	941	0.190	0.720	0.366	0.291
6H	633	0.208	0.721	0.366	0.291
7H	640	0.248	0.704	0.375	0.295
Unknown	1841	-	-	-	-
Overall	6519	0.231	0.719	0.366	0.290

[a]SNPs filtered for MAF (≤0.05) and missing data (≥10%)
[b]PIC-Polymorphic information content

The quantified genetic similarity between genotypes using Nei genetic distance (Nei 1972) resulted into two main clusters of significant size corresponding to row type. Furthermore, within the same cluster, genotypes aggregated depending on their adaptation mode (high-input barley, low-input barley or landrace). The largest distance (D=0.89) was found between AM-27 (LIMON/BICHY2000//DEFRA/DESCONOCIDA-BAR) and AM-300 (Arimont). The smallest genetic distance (D=0.00) was observed between seventeen pair of genotypes, all sister lines originated from same crosses. In order to demonstrate the phylogenetic relationships of the studied 336 barley genotypes, an Unweighted Pair-Group Method using Arithmetic averages (UPGMA) dendrogram was generated (Fig. 3) and all genotypes were assigned to two major groups (two-row or six-row barley) and three sub-groups (high-input barley, low-input barley or landraces).

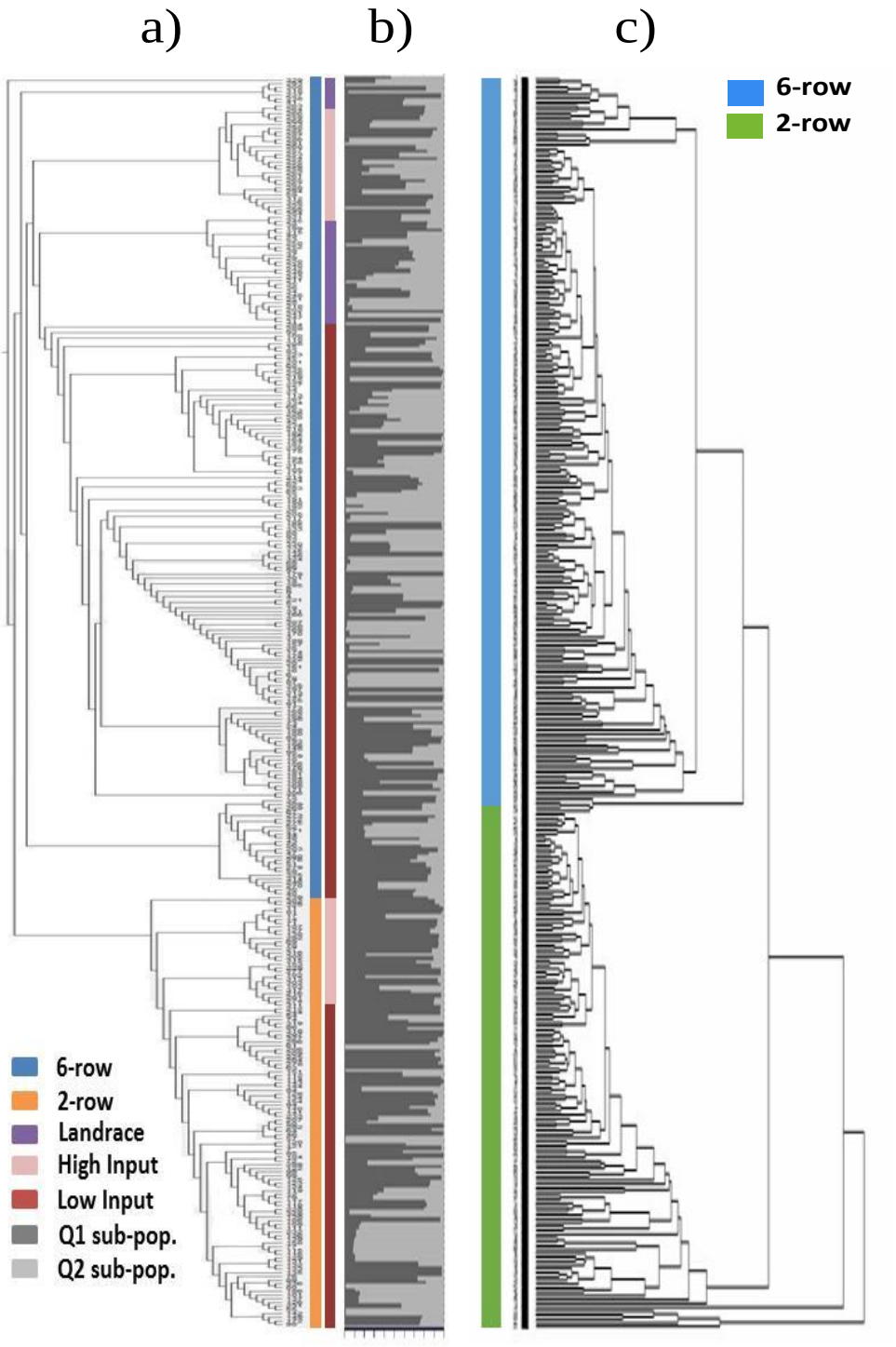

Figure 3: a) UPGMA dendrogram of 336 barley genotypes using SNP markers, b) inferred population structure based on 6519 SNPs markers and 336 barley genotypes. Each individual is represented by Q1 and Q2 sub-populations (the estimated membership fraction to each sub-population defined by STRUCTURE), c) hierarchical cluster based on phenotypic traits of 326 barley genotypes.

4.5. Population structure analysis

The break point of ΔK in the current study was K=2 (Fig. 4). As described by Evanno et al. (2005), the true value of K is when ΔK, an ad hoc quantity of the second order rate of change of the likelihood function with respect to K, reached its peak. Out of 336 genotypes, 138 (41.08%) were assigned to sub-population 1 (Q_1) and 84 (25%) were assigned to sub-population 2 (Q_2) while the remaining 114 genotypes (33.92%) were admixed (membership coefficient, $Q_i \leq 0.8$). The genetic structure of the collection was also analyzed using Principal Coordinate Analysis (PCoA). The PCoA of genetic distance revealed a clear differentiation between two and six-row barley sub-populations (Fig. 5). The first and second axes explained 45.49% and 18.05% variations, respectively, and separated genotypes in different clusters corresponding to the row type. One of the clusters mostly contained two-row while another cluster contained six-row barley genotypes. However, some overlap between two-and six-row clusters was also observed.

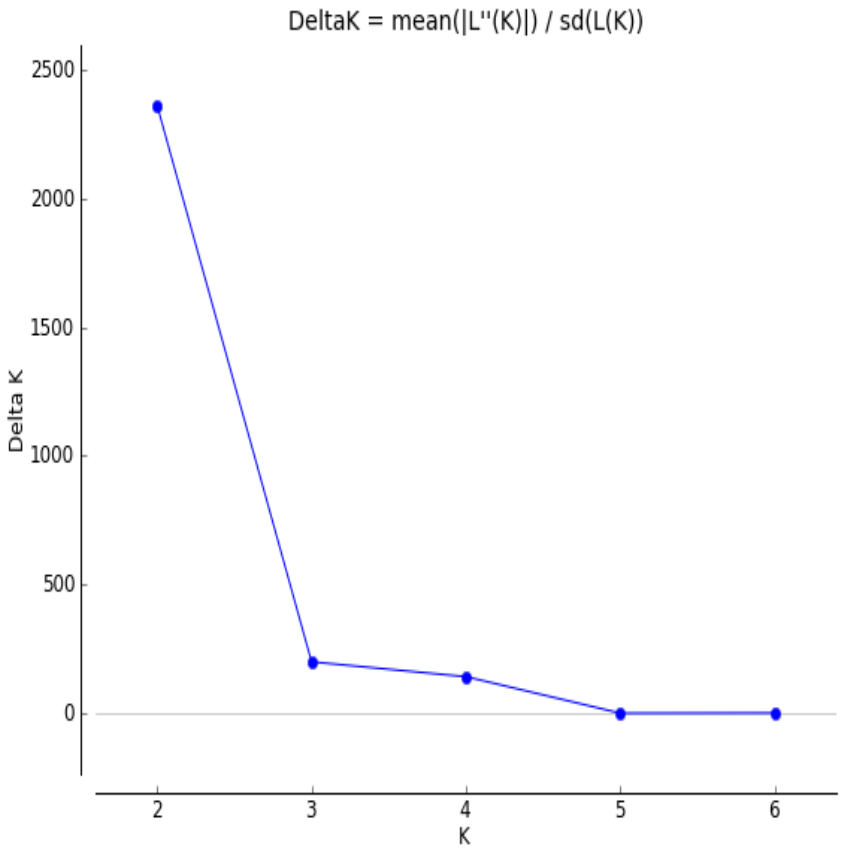

Figure 4: Population structure of AM-2014 panel using ΔK approach in STRUCTURE Harvester.

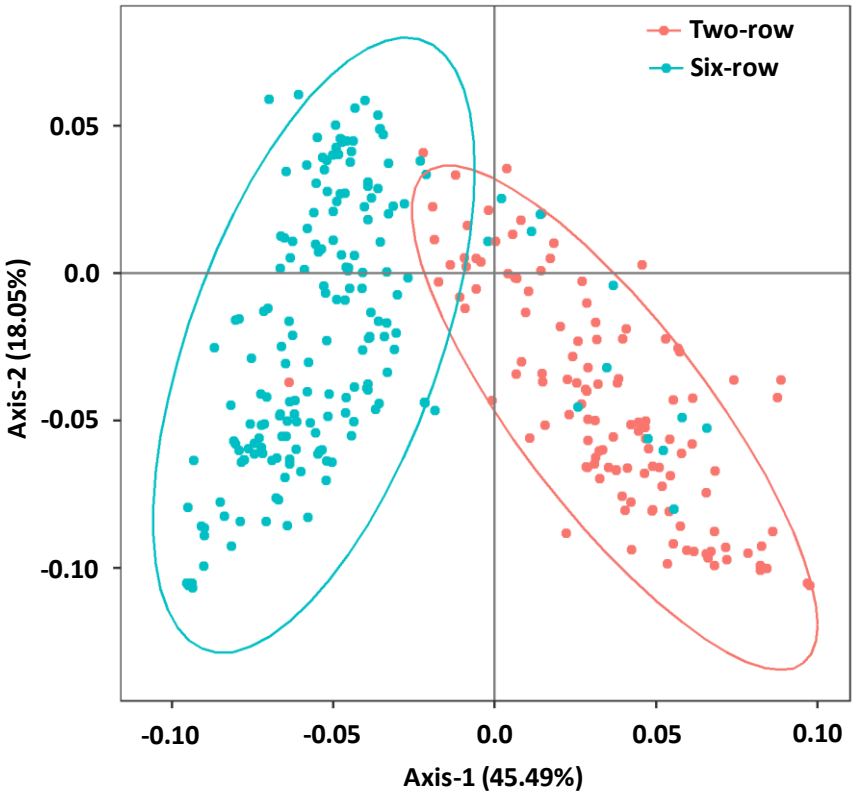

Figure 5: Principal Coordinate Analysis based on genetic distance computed for 336 barley genotypes using 6519 SNP markers genotyped for AM-2014 panel.

4.6. Linkage disequilibrium (LD)

The extent of LD was assessed among all chromosomes as well as for the two sub-populations separately. For all genotypes, 16.27% of the total SNP pairs were in LD at $P<0.001$ and 26.53% at $P<0.05$ significance. In our samples, the genome-wide LD decay was 3.58 cM at $r^2>0.15$ (Fig. 6). However, for the two-row genotypes, the number of SNP pairs that are in LD is 29.62% ($P<0.05$) and 19.65 ($P<0.001$) and for six row genotypes it is 32.78% ($P<0.05$) and 21.96 ($P<0.001$). The decay values are 3.91 cM for two-row barley and 2.26 cM for six-row barley.

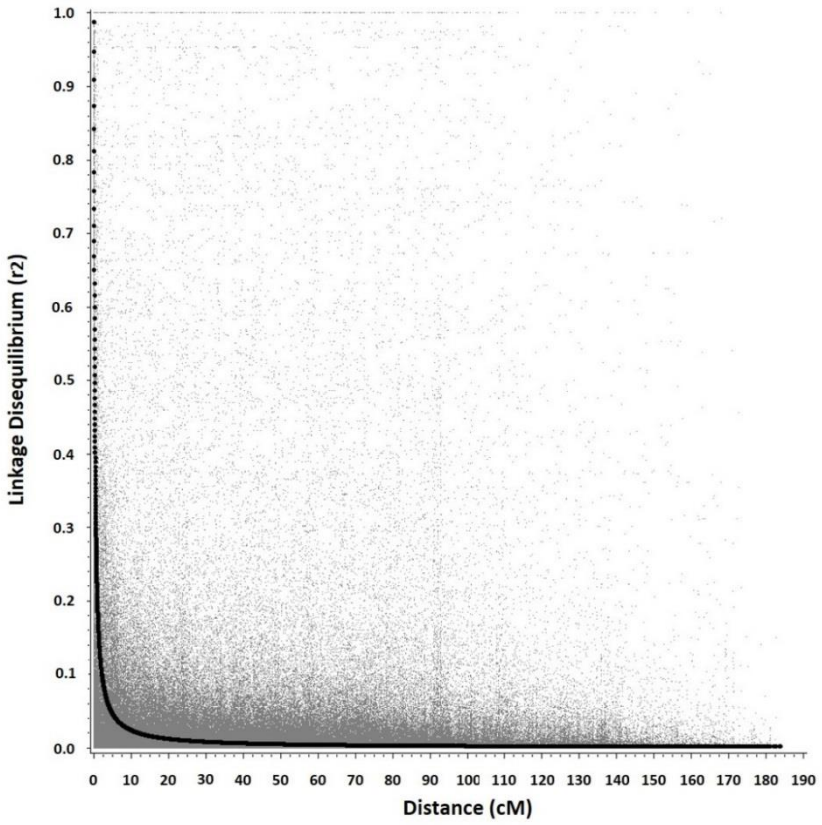

Figure 6: Linkage disequilibrium (LD) decay in 336 barley genotype collections by plotting r^2 values against genetic distance (cM)

5. Discussion

5.1. Phenotypic diversity

Descriptive statistics (mean, range and standard error of the means) of 24 agronomic traits indicated high levels of variation in barley genotypes. Based on PCA of the phenotypic traits, this barley collection was mainly clustered with respect to their disease resistance (SFNB and NFNB), number of grains per spike, and TKW. This clustering was quite evident since there was strong variability in terms of net blotch (both NFNB and SFNB) responses among the genotypes. The variation in number of grain per spike reflects the row type, but was not enough to separate our population into two groups as revealed by the SNPs markers. Thousand kernel weight (TKW) had the highest PC2 positive loading compared to rest of the traits which shows there was high variation in TKW in this collection. Most of two-row genotypes had larger grain than six-row in this study which was in agreement with previous reports (Ayoub et al. 2002; Marquez-Cedillo et al. 2000; Kajenr and Jonsen 1996). Grain weight compensates for early

stages of stresses if favorable conditions prevail during the period of grain filling. In dry areas, moisture stress is very frequent at all stages, especially grain filling. Although in many cases, the coefficients (r^2) were low, there were significant correlations among different traits. Hence a trade-off of key traits should be taken into consideration during selection and breeding.

Further, the classification of genotypes based on hierarchical clustering using Euclidean distance resulted in two main groups, six-row and two-row types. This is in support to the classification of the SNP markers. However, subgroups within a given cluster gathered with contrasting expression of agronomic traits. Based on the agronomic merit of each subgroup, the genotypes can be classified according to their disease resistance/susceptibility, biomass, yield, height and earliness. No specific differentiations can be made based on other traits. The maximum distance was found between AM-1 (Alanda/5/Aths/4/Pro/TolI//Cer*2/TolI /3/5106/6/Baca'S'/3/AC253//CI08887/CI05761), a six-row genotype, highly susceptible to NFNB, semi-dwarf with short spikes and AM-304 (CI3576) which is a two-row landrace highly resistant to NFNB, tall with long spikes. This amplitude of agronomic traits and disease resistance in barley genotypes reflects the wide genetic variability present in our collection, which is a fundamental condition for the genetic improvement. Similar observations were reported earlier by Shakhatreh et al. (2010) and Manjunatha et al. (2007) in barley collections according to agro-morphological traits.

5.2. Genetic diversity

The current study is amongst the first in ICARDA barley gene pool to deliberately assemble and analyze a specific population representing very diverse cultivated barley from ICARDA germplasm to provide a platform of GWAS for several important traits. We used SNP markers because it offers a highly polymorphic, co-dominant, and high-throughput marker system which can be used in germplasm characterization and selection of desirable alleles in breeding programs (Lombardi et al. 2014). Minor allele frequency (MAF) and expected heterozygosity are directly correlated. This additional measure can determine the proportion of rare alleles (MAF<0.2), which in turn determines the diversity of the population. In our study, we found an average expected heterozygosity of 0.29 which is comparable to that observed in other studies (Lombardi et al. 2014; Emanuelli et al. 2013; Jones et al. 2007; Ching et al. 2002). Furthermore, the average gene diversity in our sample was 0.366, which is slightly higher than reported by Rodriguez el al. (2012) and Sun et al. (2011) using SSR markers, 0.298 in barley

landraces from Sardinia and 0.338 in a worldwide barley genotypes, respectively. Higher genetic diversity in the current association mapping panel is generally expected because of the diverse nature of genotypes used (different barley breeding programs across globe, landraces collected from diverse geographical regions).

Many of the ICARDA's breeding lines, analyzed in this study, have shared common parents. As genetic distance is based on the principle that shared alleles are identical by descent, this measure of discrimination power is meaningful in case of our population. The maximum distance was found between Arimont, an American six-row, naked genotype and LIMON/BICHY2000//DEFRA/DESCONOCIDA-BAR, a two-row malt barley cross derived from highly separated localities and breeding programs and inversely, the lowest distance was found between pairs of sister lines from the ICARDA breeding programs, which is evident as they had same parentage.

5.3. Population structure and Linkage disequilibrium

Cluster analysis based on Nei (1972) distances separated, with some exceptions, the genotypes according to their row type. Our results correlate with previous studies showing a clear separation between two- and six-row types (Usubaliev et al. 2013; Chaabane et al. 2009; Chen et al. 2009; Lasa and Igartua 2001; Franckowiak and Lundqvist 1997). Historically, in ICARDA, breeders had made several two-by-six row crosses which was evident in this study by the identification of admixtures (Fig. 3b). This admixture was clearly shown from the pedigree of ICARDA barley breeding lines where both two- and six-row genotypes were included in particular crosses (Annex 1). Hence, both structure and PCA analyses support the hypothesis of genetic admixture of two- and six-row barley in ICARDA germplasm.

Despite that the optimum number of subpopulations was two (K = 2), genotypes tend to cluster (based on their coefficient of membership; *Qi*) according to their adaptation mode (high-input barley, low-input barley), regardless of their row-type. This is evident since ICARDA had two distinct barley breeding programs, in the past, located in Syria and Mexico based on target countries and end uses. The one in Syria was the low-input breeding program where the developed genotypes are more adapted to stressed environments (poor crop management, cold and drought conditions), and are bred for feed and food purposes. Whereas the genotypes developed in Mexico under the high input breeding program are more adapted to favorable

conditions (high rainfall/irrigated and appropriate crop management) and mainly bred for malt or feed. However, in the current study, the structural tendencies may not be absolute as 34%of the genotypes were admixed, and can be derived from the crosses of different parents and may be suitable for both environments.

In this study, LD at P<0.001 was observed in 16.27% of loci pairs and in 26.53% at P<0.05 significance level, where 74.4% are linked (<40 cM). Our results considerably exceeded LD reported by Rodriguez et al. 2012 using S-SAP markers where 25 genotypes of Hordeum spontaneum with 15% of loci pairs at P<0.05 and 13% of loci pairs at P<0.01 in a landrace population of Sardinia were observed. Our results were lower than the proportion reported by Malysheva-Otto et al. (2006), where 42% of loci pairs at P<0.05 in 207 European two-row spring barley using SSR markers were observed. The most plausible explanations for the moderately low LD in our collections compared to Malysheva-Otto et al. (2006) are, the use of bi-allelic SNP markers and secondly, nature of barley germplasm used in this study. Our panel includes a considerable number of landraces while breeding lines used in the current study were generated by frequently including landraces in the ICARDA's barley breeding programs. The number of detected loci pairs in LD is greater in multi-allelic markers such as SSR compared to biallelic markers such as SNPs. Also, the level of LD is higher in cultivated barley compared to landraces and wild genetic resources (Flint-Garcia et al. 2003). In the current study, we used bi-allelic SNP markers and nearly 12% of our population consisted of landraces or cultivars with a background of wild barleys, therefore an average low level of LD was expected (Massman et al. 2011; Cockram et al. 2008; Malysheva-Otto et al. 2006).

Mean r^2 LD values higher than 0.15 extended up to 3.58 cM in our study and we argued that the current marker density (1 SNP per 0.231 cM) was sufficient for genome wide association scan in barley. In case of bi-allelic markers, previous studies have reported successful association mapping in barley using a marker density of 1 DArT marker per 1.5 cM (Comadran et al. 2009) and 1 SNP marker per 0.72 cM (Pasam et al. 2012; Massman et al. 2010; Cockram et al. 2008). Furthermore, the 9K SNP platform was successfully used for various GWAS of different traits in barley (Richards et al. 2017; Wonneberger et al. 2017a; Tamang et al. 2015; Alqudah et al. 2014; Munoz-Amatriain et al. 2014).

Conclusion

This chapter provided a detailed description of a population that represents a wide range and historical survey of barley diversity within ICARDA germplasm. It comprised a considerable proportion of genetic and phenotypic variation underlying the different strategies for adaptation to different environments. We have demonstrated that the barley genotypes studied were genetically and phenotypically diverse, and strongly structured. Marker coverage, population stratification and the level of LD in our germplasm set was appropriate to run different GWAS studies for key traits in barley.

IV. Chapter 2: Genome-wide association studies of net form net blotch resistance at the seedling and adult plant stages

Abstract

Net form of net blotch (NFNB) of barley, caused by *Pyrenophora teres* f. *teres* (*Ptt*) Drechsler (anamorph: *Drechslera teres* [Sacc.] Shoem.), is considered one of the major constraints in barley production in most barley growing regions across the globe. Resistance to NFNB was evaluated in AM-2014 at seedling stage using isolates LGDPtt.19 and TD10, and adult stage in seven hotspot environments in Morocco. Genome-wide association studies (GWAS) was carried out using mixed linear model (MLM: Q+K) accounting for population structure (Q) and kinship (K) as covariates. Significant (P<0.001) marker-trait associations were corrected for false discovery rate (FDR) at the q<0.05. Four genotypes showed an average infection response (IRs≤2) to both isolates at the seedling stage, 30 genotypes showed resistance in all environments in the field and three genotypes exhibited the highest resistance at both growth stages. The GWAS of NFNB resistance identified 31 distinct QTL on all seven barley chromosomes, of which 8 were associated with resistance at seedling stage, 21 were associated with resistance at the adult stage, and two QTL, *QRptt.2H-132.15* and *QPtt.6H-54-55* conferred resistance at both stages. Of 31 resistance QTL reported in this study, 10 QTL coincided with previously mapped QTL while 21 are novel, thereby validating the GWAS approach used in this study. The resistance sources identified in AM-2014 and the QTL mapped are valuable resources for marker-assisted breeding for NFNB resistance in future.

Introduction

Net blotch (NB) of barley, caused by *Pyrenophora teres* Drechsler (anamorph: *Drechslera teres* [Sacc.] Shoem.), exists in two forms: net form of net blotch (NFNB) and spot form of net blotch (SFNB) where the causal agents are *Pyrenophora teres* f. *teres* (*Ptt*) and *Pyrenophora teres* f. *maculata* (*Ptm*), respectively. The NFNB symptoms are expressed as elongated lesions with dark brown blotches, longitudinal and transverse striations with a net like appearance (Mathre 1997; Steffenson et al. 1999), whereas SFNB symptoms consist of dark brown or elliptical lesions surrounded by chlorotic zones (Mathre 1997). The disease is considered to be destructive and economically important in many barley-growing regions around the world

(Shipton et al. 1973; Mathre 1997) and is more prevalent under cool, humid conditions but can also be found in warm and dry areas (Shipton et al. 1973). Net blotch can cause yield losses up to 40% on susceptible cultivars if disease occurs under favorable environmental conditions (Mathre 1997). Furthermore, malting and feed quality of barley is negatively affected as the infection leads to a reduction in kernel size, plumpness, and bulk density (Mathre 1997; Grewal et al. 2008). Chemical control, cultural practices, and host resistance are commonly used to manage net blotch in barley (Jordan et al. 1984, Brown et al. 1993, Youcef-Benkada et al. 1994, McLean et al. 2016), yet the most cost-effective and environmentally friendly way to control the disease would be the deployment of resistant cultivars (Robinson and Jalli 1996, Jalli and Robinson 1999). However, early work showed that the barley-NFNB genetic interaction system was complex. On the pathogen side, several studies revealed high levels of virulence diversity depending on geographical regions and selection pressure on the pathogen populations (reviewed in Liu et al. 2011). On the host side, resistance or susceptibility to NFNB can be qualitative or quantitative. The former one has been proposed to follow a gene-for-gene model (Afanasenko et al. 2007; Friesen et al. 2006). Other studies have pointed out that there is also an evidence of an inverse gene-for-gene interaction (dominant susceptibility genes), in addition to the classical gene-for-gene interaction proposed by Flor (1956) (Abu Qamar et al. 2008; Liu et al. 2011). Several studies reported dominant, incompletely or recessive genes associated with seedling or adult stage resistance to NFNB, and have concluded that resistance or susceptibility genes are present in all seven barley chromosomes (reviewed in Liu et al. 2011). However, all of these studies were done on a bi-parental populations and the identified QTLs are specific to the genotypes used and may represent interactions to narrow populations of both the host and pathogens.

Considering the limitations of bi-parental mapping to identify multiple resistance genes/QTL, we used in this study the genome-wide association mapping approach on diverse germplasm to efficiently capture marker-trait associations. Using GWAS, Richards et al. (2017) reported resistance to NFNB using three North American isolates and similarly, Wonneberger et al. (2017a) in a collection on Nordic barley germplasm. Hence, the objectives of this chapter are to report resistance to NFNB at the seedling and adult plant stages, and map QTL against *Pyrenophora teres* f. *teres* in AM-2014. The identification of marker-trait associations will facilitate the marker-assisted selection by barley breeders in order to incorporate resistances or eliminate susceptibility targets from elite material.

1. Disease screening

1.1. Phenotyping for seedling resistance (SR)

Seedlings of the AM-2014 panel were grown under controlled conditions in the greenhouse at North Dakota State University (NDSU), Fargo, ND, USA. Three to five seeds of each genotype were planted in a single cone of 3.8 cm diameter and 20 cm long, and placed into cone racks bordered with the susceptible barley cultivar, 'Robust' to eliminate edge effects. The three seedlings in each cone were evaluated as a single replicate. The barley lines 'CI 5791' and 'Tradition' were used as resistance and susceptible checks, respectively. The North American isolates, LGDPtt-19 (Ptt19) and TD10, were used to assess the infection responses of NFNB. Inoculum preparation, inoculation, and incubation used for screening seedling resistance were performed as described by Friesen et al. (2006). Infection responses were observed and recorded from the secondary leaf 7 days post inoculation in two independent replicates for each isolates using a 1-10 scale as described by Tekauz (1985).

1.2. Phenotyping for adult-plant resistance (APR)

The AM-2014 panel was sown in paired rows of 1m with 30 cm spacing of rows, 60 cm between plots and 1m between blocks. Entries were arranged using an alpha-lattice design with two replicates and each replicate accommodated 34 blocks. The experiments were conducted during the cropping season 2015 at the experimental stations of Marchouch (MCH.15), Sidi El Ayidi (SE.15), IAV-HII (IAV.15) and Jemaa Shaim (JS.15) whereas in the 2016 season, trials were conducted at Sidi El Ayidi (SE.16), Allal Tazi (AT.16) and IAV-HII (IAV.16) research stations. The natural infections were further promoted with sprinkler irrigation applied in late afternoon, when temperature and relative humidity are favorable for the disease growth at SE, AT, and IAV locations. The NFNB phenotypic reactions were assessed when disease development was sufficient on the susceptible checks, between the GS 58 (emergence of inflorescence completed) and GS 75 (medium milk) growth stages (Zadoks et al. 1974). Based on whole plots, disease screening was visually recorded using the double-digit scale (00-99) where the first digit (D1) indicates vertical disease progress of the plant and the second digit (D2) shows the disease severity (Saari and Prescott 1975; Eyal et al. 1987). Percent disease severity is estimated based on the formula: **Severity % = ((D1/Y1) x (D2/Y2) x 100)**, where Y1 and Y2 represent the maximum score on the scale (9 and 9) (Sharma and Duveiller, 2007).

Then, the genotypes were classified in six categories: Highly resistant (0-5%), resistant (5-10%), moderately resistant (10-20%), moderately susceptible (20-30%), susceptible (30-40%) and highly susceptible (>40%) (Eyal et al. 1987).

2. Phenotypic data analysis

The NFNB severity data were subject to angular transformation, then analyzed by using restricted maximum likelihood (REML) directive of GenStat 2015 (Payne 2013) to estimate genotype (G), environment (E), and G×E interaction effects when accounting for incomplete block effects of the alpha design. After finding that the blocks were not effective, we ignored them and used the standard ANOVA procedure in GenStat 2015 using following model:

$$\boldsymbol{Y_{ij}} = \mu + \boldsymbol{\tau_i} + \boldsymbol{\beta_j} + \boldsymbol{\gamma_{ij}} + \boldsymbol{\epsilon_{ijk}}$$

Y_{ij}= Net form of net blotch (NFNB) severity recorded for ith genotype in jth environment, μ = overall mean NFNB response, τ= ith genotype response, β= jth environment response, γ = genotype × environment interaction, ϵ =Error term. All statistical analyses were performed using the transformed data. All NFNB data were analysed using plot wise data from all the test locations and the variance components for genotypes (σ_g), environment (σ_e) and genotype × environment interactions ($\sigma_{g \times e}$) were estimated using the restricted maximum likelihood (REML) method. The genotype and environment effects were assumed and the interactions of genotypes with environments were assumed random.

3. Results

3.1. Phenotypic evaluation

Analyses of the phenotypic data showed a wide range of variability in disease responses at both growth stages. The ANOVA revealed a highly significant differences (P<0.001) on responses to NFNB among the genotypes (G), environments (E) and in the G×E interactions (Table 6) in field experiments. The disease severity of barley genotypes to NFNB, at the adult stage, ranged from 0 to 100% depending on the environments. The highest percentage of highly resistant (HR) genotypes, with disease score ranging from 0% to 5%, was observed (55.05%) at IAV.16,

whereas the lowest was (22.32%) at SE.15 (Table 7). Ten barley genotypes were very resistant with an average severity ≤ 5% across all environments, namely 'TR250', 'CI11456', 'NDB1173', 'TR03189', 'BICHY2000 / SHENMAINO.3', 'Keel', 'Akrash // WI2291 / WI2269 /3/ Sls / Akrash-02', 'Weeah11 // WI2291 / Bgs /3/ ER / Apm // AC253', 'QUINA / MJA // SCARLETT', and 'WI3167 /6/ ANCA / 2469 // TOJI /3/ SHYRI /4/ ATACO /5/ ALELI /7/ Schooner / Babunj // Noor68 / Kataf'. The mean infection response (IRs) at the seedling stage ranged from 1 to 9 for the isolate Ptt19 and from 1.5 to 8 for the isolate TD10 (Table 7). The proportion of genotypes showing HR response (IRs ≤2) was relatively higher against the isolate Ptt19 (12.79%) compared to the isolate TD10 (2.97%) at seedling stage. Of the 336 barley genotypes, four genotypes were found to be highly resistant to both isolates (average IRs of ≤2), namely 'WI3180 /4/ ALISO / CI3909.2 // HB602 /3/ MOLA / SHYRI // ARUPO *2 / JET', 'Harmal', 'PL172' and 'CI5791', with an average severity in the field of 5.93%, 7.94%, 8.31% and 12.87%, respectively.

Table 6: ANOVA of net form of net blotch (NFNB) resistance in 340 barley genotypes evaluated in seven environments in Morocco in 2015 and 2016.

Sources of variation	df	Mean Squares	*P*-value
Environment (E)	6	16639.15	<0.0001
Genotype (G)	335	745.36	<0.0001
G × E	1938	249.04	<0.0001
Error	2226	51.35	

Table 7: Response of barley genotypes to NFNB at the adult plant and seedling stages (seven environments in 2015 and 2016, and Ptt19 and TD10 isolates in growth chambers).

Responses to NFNB	2015				2016				
	SE	MCH	JS	IAV	SE	AT	IAV	Ptt19	TD10
[a] Number of HR genotypes	75	121	128	111	95	78	185	19	10
[c] % of HR genotypes	22.32	36.01	38.09	33.03	28.27	23.21	55.05	12.79	2.97
[b] Number of S and HS	5	9	21	20	81	70	-	10	14
[c] % of S and HS genotypes	1.48	2.68	6.25	5.95	24.10	20.83	-	2.79	4.16
[d] Range of NFNB severity	0-33.3	0-49.4	0-55.6	0-77.8	0-100.0	0-88.9	0-19.7	1-9	1.5-8
[e] LSD	4.9	1.2	16.7	18.7	2.2	20.3	7.2	11.1	18.3
[e]CV%	3.3	1.2	10.9	1.4	1.8	25.3	0.3	32.1	22.6

[a]Highly resistant (HR) genotypes were grouped when NFNB severity ranged from 0-5% (adult) or 1-2 (seedling) using angular transformed data

[b]Susceptible (S) and highly susceptible (HS) genotypes were grouped when NFNB severity ranged from 30-40% and >40%, respectively (adult) or 8-9 (seedling) using angular transformed data.

[c]Percentage was calculated out of 336 test genotypes excluding checks

[d]Range of unadjusted NFNB severity

[e]LSD and CV% were estimated using transformed data

3.2. Association mapping and identification of resistance QTL

The -$\log_{10}$ (*P*) values of significant SNP markers across the barley genome are presented in Figure 5. QTLs, significant SNPs information (marker ID, chromosome and position), phenotypic variation (R^2), allele frequencies, additive effects and the isolate or location/year are presented in Supplemental Table S2. The GWAS of NFNB identified a total of 47 significant (P <0.001) SNP markers associated with NFNB severity and IRs, among them, 12 and 35 were detected at the seedling and the adult plant stages, respectively (Table S2, Fig. 7). Minor allele frequency of significant markers ranged from 0.05 to 0.45 and R^2, phenotypic variation explained by SNPs, ranged from 3.35 to 7.01% (Table S2). The additive effects of significant SNPs were negative for 29 markers and positive for 18 markers (Table S4). Based on a cut-off of 3.6 cM genome-wide LD decay, a total of 31 unique loci (QTL) were identified on all seven barley chromosomes. In this study, three QTL on chromosome 1H (4.11, 92-93 and 125.99 cM) showed association with NFNB resistance followed by eight QTL on chromosome 2H (7.44, 40.79, 57-59, 92.21, 114-117, 126.77, 132.15 and 143.13 cM), three QTL on chromosome 3H (122.59, 144.65, and 154-155 cM), two QTL on chromosome 4H (81.57 and 97.66 cM), six QTL on chromosome 5H (43.76, 80.35, 130.03, 139.38, 143.4 and 160.49 cM), five QTL on chromosome 6H (35.62, 49.79, 54-55, 78.4 and 98.55) and four QTL on chromosome 7H (0.39, 23.02, 42.28 and 74.29 cM).

Of 31 QTL, 10 showed more than one co-segregating SNPs significantly associated with NFNB resistance (Table S2). Likewise, of 31 QTL identified in this study, 21 were found at the adult plant stage and 8 were associated with the seedling resistance against either LDGPtt.19 or TD10 isolates. Two QTL, *QRptt.2H-132.15* and *QRptt.6H-54-55* were found associated with NFNB resistance at both stages. The largest negative additive effect at any specific locus was contributed by *QRptta-3H-154-155* for the adult plant resistance to NFNB, explaining the highest phenotypic variation (7.01%). This QTL with three co-segregating SNPs, contributed up to 10.81% decrease of NFNB severity in the field. For seedling resistance, SCRI_RS_146867 (*QRptt.6H-54-55*) explained the highest phenotypic variation (5.1%) whereas the largest effect was contributed by *QPtts.5H-80.35* which was -1.42 units out of 1-10 NFNB disease scoring scale for *Ptt.19* (Table S2). Using blast search of significant SNP sequences, several annotations related to biotic and abiotic stress tolerance genes were identified Table S3.

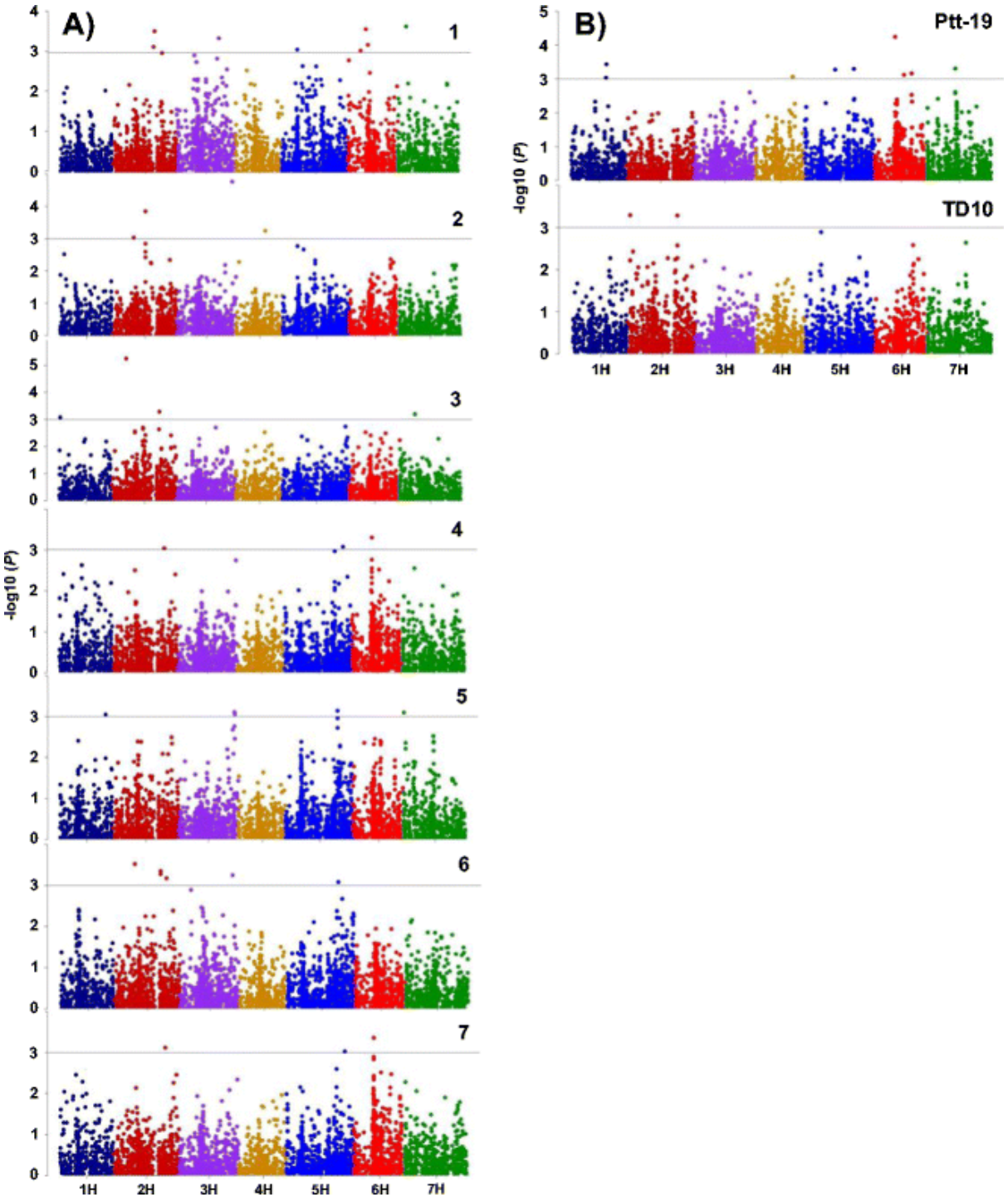

Figure 7: Genome-wide association mapping of net form of net blotch (NFNB) resistance at the **a)** adult plant and **b)** seedling stages. At **a)**, 1 = IAV-15, 2 = IAV-16, 3 = SE-15, 4 = SE16, 5 = JS-15, 6 = MCH-15, and 7 = AT-16 environments. **b)**, Ptt-19 and TD10 represent NFNB isolates originated from the USA. Colored pixels represent individual SNP markers used in the association analysis. The Manhattan plot shows $-log_{10}$ of *P* values from genome-wide scan plotted against the position of SNPs on each of the seven chromosomes. The horizontal line indicates the genome-wide significance threshold ($P < 0.001$ [$-log_{10}(P) = 3$]). All markers above the significance threshold are significantly associated with NFNB resistance.

4. Discussion

In this study, one of the most comprehensive GWAS of NFNB resistance at both the seedling and the adult plant stages has been presented. A total of 31 QTL, eight at the seedling stage alone, 21 at the adult plant stage and two QTL conferring resistance to NFNB at both stages were identified. Several novel QTL were detected in GWAS performed on the AM-2014 panel. Of the 31 QTL detected, 10 QTL namely, *QRptts-2H-7.44* (Wonneberger et al. 2017b), *QRptta-2H-40.79* (Steffenson et al. 1996), *QRptta-2H-57-59* (Cakir et al. 2011), *QRptta-2H-143.13* (Cakir et al. 2011), *QRptta-3H-118.3* (Grewal et al. 2008), *QRptts-5H-130*.03 (Konig et al. 2013; Wonneberger et al. 2017b), *QRptta-6H-49.79* (Richards et al. 2017); *QRptt.6H-54-55* (Steffenson et al. 1996; Friesen et al. 2006; St. Pierre et al. 2009; Koladia et al. 2016; Richards et al. 2017, Wonneberger et al. 2017a), *QRptts-6H-78.4* (Grewal et al. 2008) and *QPtts-6H-98.55* (Abu Qamar et al. 2008), which were previously mapped, were detected in this study. While the remaining 21 QTL were novel NFNB resistance QTL that had not been previously reported. These results demonstrated the validity and usefulness of GWAS for the identification of novel NFNB resistance from a diverse ICARDA barley germplasm collection.

Net form of net blotch (NFNB) is one of the most important barley diseases and it can cause serious damages in all barley-growing regions worldwide. Although the disease can be controlled with fungicides and good agricultural practices, the deployment of adequate resistant cultivars remains an economic and environmentally friendly approach to net blotch management. Thus, identifying new resistance genes is crucial for the development of effective and durable host resistance. Phenotypic evaluation of NFNB response in our study showed high variability among barley accessions, environments, and a strong G×E effect. Different proportions of genotypes showed high to moderate level of resistance depending on environments or the isolates used for screening (Table 7). These include a number of lines showing high level of resistance to NFNB at the adult stage in different hot spot environments and at the seedling stage against Ptt.19 and TD10 isolates, thus offering a number of potential parental lines to be used in breeding for NFNB resistance in the future. Our results are further supported by studies on molecular and phenotypic diversity of the AM-2014 where net blotch (NFNB and SFNB) was the main criteria of genotype discrimination based on PCA and hierarchical clustering (Amezrou et al. 2017).

Several genomic regions associated with NFNB resistance have been identified in previous studies through bi-parental mapping (reviewed in Liu et al. 2011). Genome-wide association studies (GWAS) is therefore an alternative approach and can overcome limitations of

traditional QTL mapping using bi-parental populations, such as time and parent selection. However, one of the major concerns while conducting GWAS remains the detection of false positives. The AM-2014 panel indicated a strong population structure based on row types. Both STRUCTURE and PCoA analyses strongly suggested two subpopulations in the AM-2014 panel, which was used in controlling false positives in GWAS. Furthermore, we have also investigated the level of LD and marker properties, which are suitable for GWAS for relevant barley traits (Amezrou et al. 2017). In order to reduce the error and avoid detection of false positives, we used the MLM: *Q+K*, accounting for population structure and kinship (relatedness) in our association studies, followed by correction of significant markers using FDR at $q<0.05$ (Patterson et al. 2006; Yu et al. 2006; Zhao et al. 2007; Wonneberger et al. 2017a). Recent studies reported the use of the GWAS approach to identify resistance QTL from a core global germplasm (Richards et al. 2017) and in a Nordic spring barley collection (Wonneberger et al. 2017a).

From the GWAS results, three QTL were found significantly associated with adult and seedling resistance to *Ptt* on chromosome 1H. Two adult-plant QTL that explained 4% of the phenotypic variation were not previously reported to be associated with NFNB resistance. Similarly, two highly significant SNP markers associated with resistance to Ptt19, mapped to the 92-93 cM region (*QRptts-1H-92-93*) had not previously been reported. The QTL explained up to 3.9% of phenotypic variation, and contributed up to -1.21 in the 1-10 scale. A strong additive effect of *QRptts-1H-92-93,* suggests that the alleles have contributed NFNB resistance, and therefore it is referred to as novel seedling QTL and we have annotated a candidate resistance gene associated with the locus using blast search.

On chromosome 2H, six QTL were associated with APR, one with seedling resistance to NFNB and one QTL was associated NFNB resistance at both growth stages (Table S2). Wonneberger et al. (2017b) identified a QTL at 7.44 cM on the short arm of chromosome 2H, associated with resistance to the Norwegian NFNB isolate 6949B in a Nordic barley collection. We identified a QTL (*QRptts-2H-7.44*) associated with resistance to TD10 at the same map position. This finding indicates that the two isolates (TD10 and 6949B) – though collected from geographically different locations – might possess common necrotrophic effectors targeting same host allele(s) at this locus. TD10 was originally collected from Montana, USA but this isolate might have shared common effectors with Norwegian isolate, 6949B. The QTL *QRptta-2H-40.79* identified in this study falls within the genomic region of the adult-plant locus mapped by Steffenson et al. (1996) in a Steptoe/Morex DH population. The QTL *QRptt.2H-132.15* was associated with adult-plant resistance, but was not found associated with resistance

to TD10 isolate. Thus, we considered *QRptt.2H-132.15* as a novel QTL. Cakir et al. (2011) mapped two QTL on the short and long arms of chromosome 2H at 62.7 cM and 140.5 cM, respectively, using Baudin/AC Metcalfe DH population challenged with the Australian isolate NB50. In this study, *QRptta-2H-57-59* (12_10948 and 11_20442) and *QRptta-2H-143.13* (SCRI_RS_159526) were significantly associated with NFNB severity in four different environments. Furthermore, these QTL are ~3 cM apart from the two QTL reported by Cakir et al. (2011), therefore, we suggest that these QTL might be same and exhibit both seedling and adult-plant resistance.

Three APR loci were mapped to the long arm of chromosome 3H in our study. Grewal et al. (2008) identified a QTL within 115-119 cM on chromosome 3H designated as *QRptta3*, explaining 5% of the phenotypic variation from a field screening of the CDC Dolly/TR251 mapping population. The QTL *QRptta-3H-118.3* falls within the genomic region of *QRptta3* and therefore the two QTL might be same QTL. The additional two QTL at 144.65 cM and 154.15 cM on chromosome 3H were not previously reported. The latter, *QRptta-3H-154.15*, with three co-segregating SNPs, explained the highest phenotypic variation (7.01%) and contributed up to 10.81% decrease of NFNB severity in the field. Therefore, this QTL could be one of the important target QTL for improving resistance to NFNB in barley in the future. One adult-plant QTL (*QRptta-4H-81.57*) mapped on chromosome 4H explained 4.42% of the phenotypic variation and was not previously reported to be associated with NFNB. Similarly, two co-segregating SNPs on the 97.66 cM of 4H, reported as QTL *QRptts-4H-97.66* were significantly associated with resistance/susceptibility to the isolate Ptt.19. These two QTL identified on chromosome 4H were never reported in previous studies, therefore we consider them as novel.

The SNP marker 12_20770 at 43.76 cM on chromosome 5H, was found to be significantly associated with adult plant resistance to NFNB and referred to as *QRptta-5H-43.76*. This locus is approximately 6 cM apart from the major SFNB resistance gene *Rpt6* (Manninen et al. 2006) suggesting that this genomic region confers resistance to both types of net blotches. In this study, two unique QTL were detected on 5H when challenged by Ptt.19 at the seedling stage. These QTL, *QRptts-5H-80.35* and *QRptts-5H-130.03*, explained 3.7% and 3.8% of the phenotypic variation, respectively. Konig et al. (2013) mapped QTL_{PV}*-5H-2* from a Post/Viresa DH population and explained 34% of the phenotypic variation. QTL_{PV}*-5H-2* is 1 cM apart from *QRptts-5H-130.03* identified in this study, suggesting that these two QTL might be same. Three additional QTL were found on the long arm of chromosome 5H at 139.38 cM, 143.4 cM and

160.49 and conferred resistance at the adult stage only. None of them was reported previously, therefore they are considered as novel QTL as well.

The chromosome 6H has long been associated with net blotch resistance (reviewed in Liu et al. 2011). The *QRptta-6H-35.62* (SNP 11_10994) explaining 3.4% of phenotypic variation is 1.88 cM apart from *SFNB-6H-33.74* reported by Burlakoti et al. (2016), suggesting that this resistance locus might contain common gene(s) conferring resistance to both forms of net blotches or might contains two distinct, yet linked genes. Various studies showed that the centromeric region of chromosome 6H harbors important NFNB dominant resistance by using diverse barley genotypes and pathogen isolates (Steffenson et al. 1996; Graner et al. 1996; Richter et al. 1998; Raman et al. 2003; Cakir et al. 2003; Ma et al. 2004; Emebiri et al. 2005; Yun et al. 2005; Manninen et al. 2000, 2006; Friesen et al. 2006; Grewal et al. 2008; Abu Qamar et al. 2008, St. Pierre et al. 2010; Richards et al. 2017; Wonneberger et al. 2017a). Predictably, we have also mapped four distinct QTL in the same genomic region within the interval of ~49-98 cM. Richards et al. (2017) identified two loci at the approximate position of 49 and 54 cM, associated with NFNB resistance/susceptibility to three *Ptt* isolates (6A, 15A and LDNH04Ptt19). Further, they found 17 marker-trait associations significantly associated with reaction to three *Ptt* isolates (6A, 15A and LDNH04Ptt19) in the ~49 cM region of chromosome 6H. We have also identified a QTL at the exact genomic region associated with adult-plant resistance, indicating that this locus is effective at both growth stages. Further, *QRptt.6H-54-55* was associated with seedling and adult-plant resistance with four significant SNPs explaining up to 5.1% of phenotypic variations. The QTL also includes the SNP SCRI_RS_140091, which is the most significant marker detected by Koladia et al. (2016) from a CIho5791/Tifang recombinant inbred mapping population, challenged by a global collection of nine *Ptt* isolates from diverse geographical regions. Similarly, Richards et al. (2017) detected 33 significant marker-trait associations in the ~55 cM region, associated with reactions to the three American isolates. More interestingly, the isolate LDNH04Ptt19 was common to the Koladia et al. (2016), Richards et al. (2017) and the present studies. Therefore, detection of same QTL was expected and validated our GWAS approach to map NFNB resistance QTL in AM-2014 panel. Collectively, these findings strongly indicate that this region, ~55 cM, harbors major NFNB resistance genes. An additional locus, *QRptts-6H-78.4* (SNP: SCRI_RS_1937) coincides within the *QRpt6* interval 75-78 cM, a major seedling and adult-plant resistance QTL, effective against both forms of net blotches (Grewal et al. 2008). Besides this, a NFNB seedling resistance locus *QRptts-6H-98.55* (SCRI_RS_155564) is located ~1.75 cM from *rpt.r*/*rpt.k*, the major susceptibility genes which were hypothesized to confer susceptibility to *Ptt* isolates 6A

and 15A (Abu Qamar et al. 2008). This result indicates that the locus *QRptts-6H-98.55*, might likely be the same QTL as *rpt.r*/*rpt.k*.

One adult-plant resistance locus identified near the telomere region of the short arm of chromosome 7H (*QRptta-7H-0.39*) was not previously reported. However, Tamang et al. (2015) identified a marker-trait association in the same genomic region, significantly associated with the SFNB isolate DEN 2.6 (SNP: 11_21419, 0.00 cM). This suggests that the loci might contain two distinct genes or common resistance alleles, associated with both forms of net blotches. Identification of the gene(s)/QTL effective for both forms of net blotches has significant implication to barley breeding in future. In addition, three novel loci detected at ~23 cM, ~42 cM and ~74 cM were specific to either seedling or adult stage resistance in this study (Table S2, Fig. 7) and were not reported previously.

Conclusion

Overall, a total of 31 genomic loci associated with resistance at either the seedling or the adult plant growth stages were detected in this study. The majority of the QTL mapped in this study were specific to one of the two isolates or the pathogen populations used for screening. This is not surprising as the specificity of the pathogen isolate or race can also be a feature of quantitatively inherited disease resistance, unlike the broad-spectrum QTLs (Young et al. 1996; Marcel et al. 2008; Poland et al. 2008; St. Clair et al. 2010). Many QTL mapping studies employing multiple pathogen isolates/races showed that isolate/race specificity can be detected as significant genotype by isolate/race interaction in an analysis of variance (Young et al. 1996; Caranta et al. 1997; Li et al. 2006), which is the case in our study (Table S3). The phenotypic variance in this case can be explained by a minor-gene for minor-gene interaction, where minor effect virulence genes in the pathogen correspond to resistance genes of minor effect in the host (Poland, 2009). In this view, knowledge of host-pathogen interactions should help barley breeders in selecting effective resistance genes in advanced breeding lines. On the other hand, haplotype analysis of significant SNP at each QTL of the highly resistant barley lines in this study should provide a useful resource for MAS for single QTL, multiple QTLs (pyramiding) or minor QTLs plus deployment of durable NFNB resistance genes. The enrichment of resistance alleles and elimination of alleles contributing susceptibility is key to the success of future NFNB resistance breeding programs in barley.

V. Chapter 3: Genome-wide association studies of spot form of net blotch resistance at the seedling and adult plant stages

Abstract

Spot form of net blotch (SFNB) of barley, caused by *Pyrenophora teres* (Drechslera) f. *maculata* is an important barley disease. If not controlled, it can cause significant yield losses in many barley production across the globe. Resistance to SFNB was evaluated in AM-2014 at seedling stage using isolate FGO, and at adult stage at seven environments in Morocco. Genome-wide association studies (GWAS) was carried out using mixed linear model (MLM: *Q+K*) accounting for population structure (Q) and kinship (K) as covariates. Significant ($P<0.001$) marker-trait associations were corrected for false discovery rate (FDR) at the $q<0.05$. A total of 22 genotypes were highly resistant in at least five environments and exhibited an IR≤2 to FGO isolate at the seedling stage. Association mapping analysis of SFNB resistance showed 21 distinct QTL on all seven barley chromosomes, of which two were associated with resistance at seedling stage, 18 were associated with resistance at the adult stage, and one QTL, *QRptm-7H-70-71* conferred resistance at both stages. Of 21 resistance QTL reported in this study, 12 QTL coincided with previously mapped QTL while nine are novel and represent a valuable resource for marker-assisted breeding for SFNB resistance in future.

Introduction

Spot form of net blotch (SFNB) caused by *Pyrenophora teres* (Drechslera) f. *maculata* (*Ptm*) is an important foliar disease of barley on crops grown USA, Canada, Australia, Scandinavia, Hungary, South Africa and the Mediterranean region (Mclean 2011). Spot form of net blotch symptoms can be distinguished from net form of net blotch by dark brown or elliptical lesions surrounded by chlorotic zones (Mathre 1997) whereas in NFNB, symptoms are expressed as elongated lesions with dark brown blotches, longitudinal and transverse striations with a net like appearance (Mathre 1997).

Though SFNB epidemics are emerging compared to NFNB, yield losses due to SFNB have become increasingly severe in recent years (Liu et al. 2011). For instance, the annual losses due to SFNB in Australia was estimated to 192 million AUS$ (Murray and Brennan 2010).

The use of resistant varieties is the most economical way to control the disease and thus, identification of SFNB resistance sources and introgression of resistance genes into elite lines is a high priority. QTL conferring seedling and adult plant resistance to SFNB have been mapped on all seven barley chromosomes (Cakir et al. 2011; Friesen et al. 2006; Grewal et al. 2008, 2012; Manninen et al. 2006; Molnar et al. 2000; Williams et al. 1999, 2003). However, all of these studies were reported using bi-parental mapping populations. Recently, genome-wide association mapping has been successfully applied to identify markers tightly linked with resistance or susceptibility to SFNB in four two-rowed Australians barley breeding populations (Wang et al. 2015), in a subset of the barley core collection (Tamang et al. 2015) and in a barley collection from the breeding programs in the Upper Midwest of USA (Burkaloti et al. 2016). However, only the study from Wang et al. (2015) reported the use of AM to identify markers associated with SFNB adult plant resistance. The main objectives of this study were to evaluate AM-2014 for the seedling and adult plant resistance to SFNB and identify the *Ptm* resistance or susceptibility loci using GWAS approach.

1. Disease screening

1.1. Phenotyping for seedling resistance (SR)

Three seeds of each accession were planted in single cones (3.8 cm in diameter and 20 cm long) and grown under controlled conditions in the greenhouse at NDSU, Fargo, USA. The cones were then placed into racks bordered with the susceptible barley cultivar 'Tradition'. The three seedlings in each cone were evaluated as a single replicate. The barley lines 'FCN 119' and 'Tradition' were used as resistant and susceptible checks, respectively. The isolate FGOB10Ptm-1 (FGO) was used to assess the barley lines for SFNB disease reaction. Inoculum preparation, inoculation and incubation used for screening seedling resistance were performed as described by Neupane et al. (2015). Disease rating was recorded on second leaf 7 days after inoculation in the two independent replicates using the 1 to 5 scale as described by Neupane et al. (2015). Barley genotypes with an infection response (IR) $\leq$1.5 were considered highly resistance.

1.2. Phenotyping for adult-plant resistance (APR)

Similarly to NFNB screening, 10g of seeds for each entry were planted in paired rows plots of 1m with 30 cm spacing, 60 cm between plots and 1m gap between blocks. The experiments were conducted in alpha-lattice design with two replications in seven environments: MCH.15, JS.15, SE.15, SE.16, IAV.15, IAV.16 and AT.16. In SE AT and IAV locations, natural SFNB infections were further promoted with sprinkler irrigation applied in late afternoon, when temperature and relative humidity are favorable for disease growth. Genotype reactions were assessed when SFNB epidemics were developed sufficiently on susceptible checks between GS 58 and GS 75 growth stages (Zadoks et al. 1974). Like NFNB, Disease screening was visually recorded based on whole plots, using the double-digit scale (00-99 and the percent disease severity was estimated using the following formula: Severity % = ((D1/Y1) x (D2/Y2) x 100), where Y1 and Y2 represent the maximum score on the scale (9 and 9) (Sharma and Duveiller, 2007). Genotypes were then classified as highly resistant (0-5%), resistant (5-10%), moderately resistant (10-20%), moderately susceptible (20-30%), susceptible (30-40%) and highly susceptible (>40%) (Eyal et al. 1987).

2. Phenotypic data analysis

The SFNB severity data was transformed using the angular transformation and analyzed by using restricted maximum likelihood (REML) directive of GenStat 2015 (Payne 2013) to estimate genotype (G), environment (E), and G×E interaction effects when accounting for incomplete block effects of the alpha design using the same model used in NFNB analysis.

3. Results

3.1. Phenotypic evaluation

At adult plant stage, ANOVA revealed highly significance differences (P<0.001) on responses to SFNB among genotypes (G), environments (E) and G×E interactions (Table 8). Disease severity of barley genotypes ranged from 0 to 77.78% depending on the environment (Table 9). The highest percentage of HR (≤5% disease severity) genotypes was recorded at IAV location in both seasons (92.26% in 2015 and 95.5% in 2016), whereas the lowest (24.7%) was at SE.16 (Table 9). The mean infection response (IRs) to the FGO isolate ranged from 1 to 3.75 and the proportion of HR (IRs ≤1.5) genotypes was 9.82% (Table 9). Among all accessions,

22 were highly resistant in at least five environments and exhibited an IRs ≤1.5 to the FGO isolate. Three genotypes namely, AM-241 (Petunia-1), AM-282 (Viringa'S//WI2291/WI2269/3/H.spont.38-3/Akrash-01) and AM-133 (Weeah11//WI2291/Bgs/3/ER/Apm//AC253) were highly resistant across all seven environment and had an infection response of 1.5, 1.75 and 1.75, respectively, to FGO isolate.

Table 8: ANOVA of spot form of net blotch (SFNB) resistance evaluated in seven environments in Morocco in 2015 and 2016.

Sources of variation	df	Sum of Squares	*P*-value
Environment (E)	6	70581.69	<0.0001
Genotype (G)	335	63238.26	<0.0001
G × E	2010	195877.96	<0.0001
Error	2260	70596.66	

Table 9: Response of barley genotypes to SFNB at the adult plant and seedling stages (FGO isolate)

Responses to SFNB	2015				2016			
	[a] SE	MCH	JS	IAV	SE	AT	IAV	FGO
[b] Number of HR genotypes	270	182	153	310	83	147	321	33
[d] % of HR genotypes	83.05	54.16	45.53	92.26	24.70	43.75	95.5	9.82
[c] Number of S and HS	8	9	2	-	4	-	-	4
[d] % of S and HS genotypes	2.38	2.68	0.6	-	1.19	-	-	1.19%
[e] Range of SFNB severity	0-77.78	0-77.78	0-32.72	0-14.81	0-38.9	0-23.77	0-9.57	1-3.75

[a] SE-Sidi El Ayedi Research Station, INRA-Maroc; MCH-Marchouch Research Station, ICARDA-Morocco; JS-Jemaa Shaim Research Station, INRA-Maroc; IAV-Institute of Agronomy and Veterinary medicine Hassan II, Rabat, Morocco; AT-Sidi Allal Tazi Research Station, INRA-Maroc

[b]Highly resistant (HR) genotypes were grouped when SFNB severity ranged from 0-5% (adult) or ≤1.5 (seedling) using angular transformed data

[c]Susceptible (S) and highly susceptible (HS) genotypes were grouped when SFNB severity ranged from 30-40% and >40%, respectively (adult) or ≥3.5 (seedling) using angular transformed data.

[d]Percentage was calculated out of 336 test genotypes excluding checks

[e]Range of unadjusted SFNB severity

3.2. Association mapping and detection of resistance QTL

Association mapping analysis was done for adult plant resistance (APR) and for seedling resistance (SR) separately. The transformed mean reactions from replicates was used as the phenotypic response input. A threshold of *P* value < 0.001 followed by a correction for multiple

testing using the FDR criterion (*q* value <0.05) was applied to identify significant SNPs associated with SFNB resistance or susceptibility loci from the MLM: *Q+K* analysis.

For the entire data set, 46 SNPs located on all the seven barley chromosomes were associated with APR (43 SNPs) and SR (3 SNPs) to spot form of net blotch, respectively (Table S4, Fig. 8). For all significant markers, *P* values ranged from 9.92E-04 to 2.13E-05, MAF had a range of 0.05 to 0.46 and the phenotypic variation (R^2) was up to 6.15% (Table S4). Based on a cut-off 3.6 cM, genome-wide LD decay, 21 QTL were found to be associated with disease resistance to SFNB, of which 18 associated with adult-plant stage, two with disease reaction to FGO isolate and one QTL was significantly associated with both growth stages. Of the loci associated with SFNB resistance or susceptibility, one was identified on chromosome 1H (*QRptma-1H-4.11*), four on 2H (*QRptma-2H-40.79, QRptma-2H-91-94, QRptma-2H-107.37, QRptma-2H-132.15*), three on 3H (*QRptma-3H-50-51, QRptma-3H-104.75, QRptma-3H-120-124*), three on 4H (*QRptma-4H-24.48, QRptms-4H-51.4, QRptma-4H-81.57*), four on 5H (*QRptma-5H-46-47, QRptma-5H-98.54, QRptma-5H-143-144, QRptms-5H-168.89*), three on 6H (*QRptma-6H-2.62, QRptma-6H-65-67, QRptma-6H-86.97*) and three on 7H (*QRptma-7H-3.82, QRptma-7H-23.02, QRptm-7H-70-71*). With few exceptions, most of the QTL identified were specific to a single environment. Seven loci were supported by more than one SNP marker, the most consistent with 16 co-segregating SNP markers at 70-71 cM (*QRptm-7H-70-71),* associated with disease reaction at both growth stages. The largest allele effect for adult plants was contributed by the marker 12_20326 (*QRptma-2H-40.79*) with a negative additive effect of 8.45% disease severity on this specific loci. In case of seedlings, the highest effect was contributed by the marker 12_31481 (*QPtms-5H-168.89*), with 0.26 units on the 0-5 scale of the FGO isolate response.

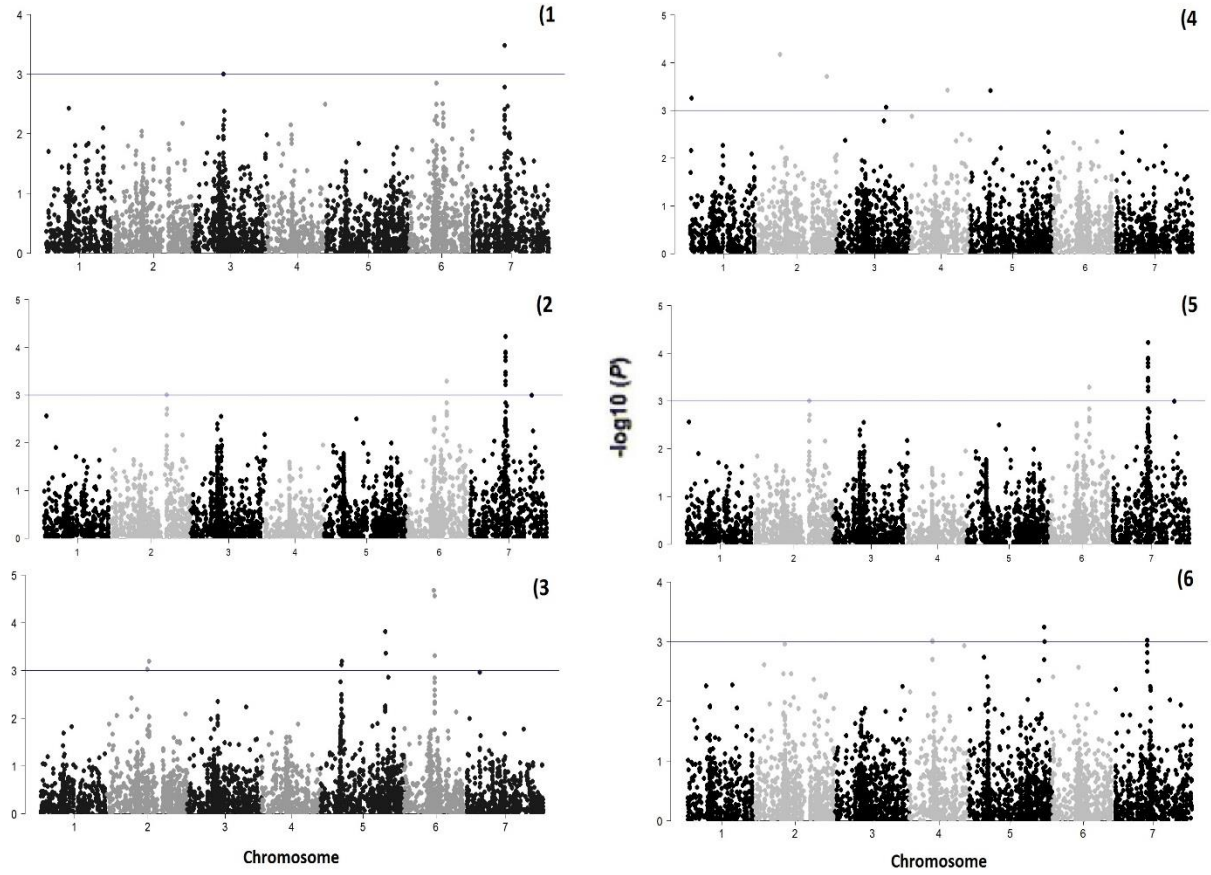

Figure 8: Genome-wide association mapping of spot form of net blotch (SFNB) resistance at the adult plant and seedling stages. 1 = IAV-15, 2 = IAV-16, 3 = SE-15, 4 = SE-16, 5=AT-16, 6=FGO isolate. Colored pixels represent individual SNP markers used in the association analysis. The Manhattan plot shows $-log_{10}$ of *P* values from genome-wide scan plotted against the position of SNPs on each of the seven chromosomes. The horizontal line indicates the genome-wide significance threshold ($P < 0.001$ [$-log_{10}(P) = 3$]). All markers above the significance threshold are significantly associated with SFNB resistance.

4. Discussion

Spot form of net blotch is an important barley disease, and along with Net form of net blotch, can cause serious damages in all barley growing regions worldwide. Although the disease can be controlled with fungicides and good agricultural practices, host resistance remains the economically and environmental friendly option to manage SFNB. Until now, several major and minor genes associated with SFNB resistance have been identified in previous studies using bi-parental mapping (reviewed by Liu et al. 2011) and recent association genetics studies (Wang et al. 2015; Tamang et al. 2015; Burkaloti et al. 2016). Because SFNB undergoes frequent sexual recombination, there is a high risk of increased virulence within the pathogenic

populations due to natural selection. Thus, identifying new sources of resistance is important for developing highly resistant lines which combine multiple resistance loci. In this study, we applied AM approach using a mixed linear model accounting for population structure and kinship matrix, followed by FDR correction of significant markers, in order to minimize false positives and to detect true marker-trait associations (Yu et al. 2006; Zhao et al. 2007; Patterson et al. 2016).

The phenotypic variation reported in this study showed a high variability in SFNB resistance among barley lines in the field and in response to the FGO isolate. Among the 336 barley genotypes in this study, 22 were highly resistant in at least five environments and exhibited an IRs ≤1.5 to the FGO isolate. These lines could be good sources for SFNB resistance in barley breeding programs.

In this study, we found 21 QTL tightly linked with resistance or susceptibility on all seven barley chromosomes, associated with SFNB adult plant and seedling resistance in a diverse panel of germplasm. Of the identified QTL, 12 correspond to previously known QTL involved in resistance to SFNB, whereas the remaining nine appeared to be novel, and may be useful for incorporating new resistance sources to SFNB in barley breeding programs. However, majority of the QTL mapped in our study were specific to a given environment and the variability of the infection responses of the genotypes, indicates the quantitative nature of SFNB resistance in the current association mapping panel. The quantitative nature of resistance for SFNB infection was also reported in previous studies (Williams et al. 1999; Friesen et al. 2006; Wang et al. 2015; Tamang et al. 2015; Burkaloti et al. 2016). This can be explained also by a minor-gene for minor-gene interaction, where minor effect virulence genes in the pathogen correspond to resistance genes of minor effect in the host, due the specificity of the pathogen isolate or race used for screening (Poland 2009).

One QTL associated with adult-plant resistance was identified on chromosome 1H, *QRptma-1H-4.11,* explained 3.62% of the phenotypic variation. The same QTL (*QRptt-1H-4.11*) was found to be associated with net form of net blotch in a previous association mapping study, suggesting that this locus is effective against both forms of net blotch (Amezrou et al. 2018). On chromosome 2H, *QRptma-2H-40.79* (the largest allelic effect QTL) is ~2cM away from *SFNB-2H-38.03*, a QTL mapped by Burkaloti et al. 2016, from a combined population of four barley breeding programs in the Upper Midwest of the USA, challenged with SFNB isolates collected from Montana, USA. Similarly, *QRptma-2H-132.15*, is located at ~2cM and ~5cM

distance, respectively, from the QTLs mapped by Cakir et al. 2011 in a Baudin/AC Metcalfe DH population using NB320 isolate and a diverse sample of the BCC (Barley core collection), challenged with the isolate FGO (Tamang et al. 2015). The SNP markers SCRI_RS_170162 and SCRI_RS_157097 (*QRptma-2H-91-94*) are predicted to encode an unknown protein and LRR receptor protein kinase, respectively. These two markers explained 3.41 and 3.64% of the phenotypic variation, respectively. This QTL was not previously reported and may be the genes containing these SNP can be considered as candidate host susceptibility or resistance genes. Using the New Zealand isolate NZKF2, Tamang et al. (2015) detected two marker-trait associations on chromosome 3H at 43.52 cM and 103 cM. This is likely to be same QTL as the *QRptma-3H-50-51* and *QRptma-3H-104.75* identified in this study, suggesting that these two QTL may be the same and confer resistance at both developmental stages.

Three marker-trait associations were found significant in chromosome 4H. The QTL *QRptma-4H-24.48* falls in the range of *QRptms4*, a QTL mapped by Grewal et al. 2012 using the CDC Bold/TR251 double haploid population and the SFNB isolate WRS857. Similarly, *QRptms-4H-51.4* resides within the net blotch resistance locus *QRpts4*, which explained up to 21% of the phenotypic variation (Grewal et al. 2008). The remaining QTL (*QRptma-4H-81.57*) was not previously reported to be associated with SFNB resistance or susceptibility. Interestingly, it was also determined that this QTL was associated with resistance to net form of net blotch and represents a potential source of resistance to two closely but distinct pathogens (Amezrou et al. 2018). The adult-stage QTL *QRptma-5H-46-47* was detected in two environments with four significant SNPs, indicating that these markers are linked together and co-segregating for SFNB resistance. This QTL may be same as *Rpt6*, a major SFNB resistance gene located at about 38 cM on 5H (Manninen et al. 2006). The remaining two QTL mapped on 5H (Table S4) were not previously reported and therefore are consider novel.

Chromosome 6H has long been associated with net form and spot form of net blotch resistance/susceptibility. The SNP SCRI_RS_199940 located at 2.62 cM on 6HS showed significant association with SFNB resistance and explained 3.39% of R^2. Burkaloti et al. 2016 also reported a QTL on the same genomic region (*SFNB-6H-5.4*). Similarly, *QRptma-6H-65-67* and *QRptma-6H-86.97* were previously reported at the seedling stage (Tamang et al. 2015) and at both growth stages (Wang et al. 2015), respectively.

The *Rpt4* gene on chromosome 7H was the first SFNB resistance gene described in the cultivar Galleon and flanked by the RFLP markers Xpsr117D and Xcdo673 at approximately 6 to 25

cM (Williams et al. 1999). We identified one locus at the *Rpt4* region (*QRptma-7H-23.02*) that explained 5.50% of the phenotypic variation at adult-plant stage. Further, the most consistent QTL detected in our study, effective at both growth stages, had 16 significant marker-trait associations at ~70-71 cM. This indicates that underlying this region is likely a cluster of SFNB resistance or susceptibility genes. Wang et al. (2015) found that the QTL with the largest effects were located on chromosome 7H. Our findings also suggest that 7H harbors several alleles of resistance and they should be accumulated to breed high-level resistance cultivars.

Conclusion

We detected most of the major and minor SFNB resistance QTL previously reported and identified major resistance genes on chromosome 2H, 3H, 4H, 6H and 7H (Steffenson et al. 1996; Williams et al. 1999; Williams et al. 2003; Cakir et al. 2011; Grewal et al. 2012; Tamang et al. 2015; Wang et al. 2015; Burkaloti et al. 2016) and new QTLs on all seven barley chromosomes. The loci identified in this chapter could harbor either resistance or susceptibility targets. Breeding strategies must combine multiple loci, either by lacking host susceptibility targets or harboring resistance loci. It is therefore important to dissect host-pathogen genetic interactions and the genes/loci conditioning lack of susceptibility and resistance for an effective deployment of SFNB resistant lines. Also, the marker haplotype analysis of the significant SNP at each QTL of the highly resistant barley lines should provide a useful resource for marker-assisted selection. This results provide important genetic information for the effective deployment of resistance or the elimination of host susceptibility factors from elite barley lines, providing a durable means of management for this important barley disease.

VI. Chapter 4: Genome-wide association studies of agronomic traits in barley

Abstract

Dissecting the genetic architecture of important agronomic traits is key in barley improvement. In this chapter we deploy the Genome-wide association studies to detect QTLs associated with days to heading, plant height, spike length, grain yield and 1000-kernel weight was deployed in the association mapping panel AM-2014. The agronomic traits were recorded in four environments and marker-trait analyses were carried out using a mixed linear model corrected for population structure, genotype relatedness (MLM: $Q + K$). Significant ($P < 0.001$) marker trait associations were corrected for false discovery rate (FDR) at the $q < 0.05$. A total of 88 QTL were detected for the five traits of which 53 are novel. The newly identified QTL are valuable resources for marker-assisted and genomic selection of useful alleles for breeding high-yielding barley cultivars.

Introduction

Understanding the genetic basis of complex agronomic traits is one of the most challenging and important objectives in crop improvement. Most of key agronomic and developmental traits are quantitative in nature, and usually are under the control of major and minor QTL, resulting in greater difficulty for dissecting the genetic architecture underlying the phenotype of interest.

Yield is the most important agronomic trait in cereal breeding. Grain yield is a complex trait with low heritability and is the compound of multiple interacting component traits and is strongly influenced by the environment (Jiang et al. 2004, Zhao et al. 2016). Also, plant developmental genes like vernalization (*Vrn-H1* and *Vrn-H2*), flowering time (*Ppd-H1*) and height (*denso, uzu*) have been reported to have direct effect on yield and yield components (Thomas et al. 1991; Hackett et al. 1992; Backes et al. 1995; Li et al. 2005; Thomas et al. 1995; Wang et al. 2010). More importantly, optimization of flowering time and plant height lead to significant yield improvements in cereal breeding (Snape et al. 2001, Cockram et al. 2007, Hedden 2003, Nadolska-Orczyk et al. 2017). Optimal flowering time allows optimal grain

development, while semi-dwarf cereals allocate more resources into grain production and show reduced losses through lodging. Major genes controlling flowering time and semi-dwarfism have been identified and the best alleles are tending to become fixed in modern breeding germplasm (Jung and Muller 2009; Jia et al. 2011). A large number of QTL mapping studies have been conducted on agronomic traits in barley using bi-parental mapping. A detailed summary of agronomic QTL placed on the consensus map of Rostoks et al. (2005) is available at the following link: http://wheat.pw.usda.gov/cgi-bin/graingenes/report.cgi?class=mapdata;name=Barley%2C%20Agronomic%20QTL%20Consensus;show=qtl. Recently, GWAS have been successfully employed on agronomic traits in barley (Cockram et al. 2010; Comadran et al. 2008, 2011; Massman et al. 2010; Ramsay et al. 2011; Roy et al. 2010; Wang et al. 2011, 2012; Pauli et al. 2014). Agronomic traits are evaluated and used in selection on a continual basis in most breeding programs, the objective of this chapter is to determine the genomic regions associated with seven key agronomic traits and the markers associated with these key traits. Efficient marker-assisted and genomic selection for these traits could accelerate the development of new barley lines.

1. Phenotyping of agronomic traits

Data of seven agronomic traits was collected from five environments (MCH.15, JS.15, SE.16 and AT.16). All the traits were recorded according to the barley descriptor (IPGRI, 1994). Days to heading (DH) was scored in days from the date of sowing until 50% of the plants in the plot have reached GS53 stage, which is a stage where one third of ear emerges from the plant. Plant height (PH) was measured in cm from the bottom to the ear tip, excluding the awns. Spike length (SL) was measured in cm from the spike tip to the base of the spike (excluding the awns). After maturity, four rows from each plot were manually harvested, excluding the border rows to avoid the grain mixture if using the combine harvester. The harvested plot is threshed and two more traits were measured: grain yield (GY) and 1000 kernel weight (TKW).

2. Association mapping of agronomic traits

2.1. Days to heading (DH)

A total of 23 SNP markers was found to be significantly (P<0.001) associated with DH in different environment (Table 10). The percentage to the phenotypic variation (R^2) was up to

4.72% (*DH.4H-38.74; DH.5H-87.36* and *DH.7H-76.47*). Using the cutoff 3.6 cM LD decay, 15 QTLs have been identified across all seven chromosomes, including the QTL *DH.2H-19.9* with SNP markers Bk_12 and Bk_16 at 19.9 cM on chromosome 2H, significantly associated with days to heading. These markers in iSelect were derived from *PpdH1*, the major heading date gene (Turner et al. 2005). Two QTLs, *DH.5H-44-47* and *DH.7H-76.47* where significant in two environments, whereas the rest was specific to a single environment (Table 10).

Table 10: Summary of QTL associated with days to heading (DH)

QTL	SNP	Chr	cM	*P*-value	R^2	Env.	Reference gene
DH.1H-72.38	11_20990	1	72.38	1.89E-04	4.29	SE16	*vrs3*
DH.2H-19.9	BK_12	2	19.9	8.87E-04	3.35	MCH15	*Ppd-H1*
	BK_16	2	19.9	8.87E-04	3.35	MCH15	
DH.2H-58.78	12_10474	2	58.78	6.31E-04	3.58	SE16	
	12_31020	2	64.83	4.53E-04	3.73	MCH15	*HvFT4, eam6*
DH.2H-64.83							
DH.2H-82.08	11_11058	2	82.08	1.89E-04	4.29	SE16	
DH.3H-51-53	11_11530	3	51.77	1.89E-04	4.29	SE16	
	11_10005	3	53.26	3.18E-04	3.98	SE16	*HvGI*
DH.4H-38.74	11_21397	4	38.74	9.18E-05	4.72	SE16	
DH.4H.51-54	12_30684	4	51.56	4.53E-04	3.73	MCH15	
	11_10509	4	55.28	6.43E-04	3.53	MCH15	
DH.5H-34.24	11_20729	5	34.24	1.89E-04	4.29	SE16	*HvCO3*
DH.5H-44-47	SCRI_RS_134444	5	44.38	6.30E-04	3.55	MCH15	
	11_21260	5	46.53	1.89E-04	4.29	SE16	
	SCRI_RS_147462	5	47.15	9.77E-04	3.33	SE16	
DH.5H-87.36	11_11350	5	87.36	9.18E-05	4.72	SE16	
DH.6H-2.62	11_21521	6	2.62	1.89E-04	4.29	SE16	
DH.6H-49.79	12_10199	6	49.79	1.89E-04	4.29	SE16	
DH.6H-91.71	11_10734	6	91.71	7.34E-04	3.49	SE16	
DH.7H-76.47	12_30565	7	76.47	9.22E-04	3.32	MCH15	
	11_11239	7	76.47	9.18E-05	4.72	SE16	

2.2. Plant height (PH)

Twenty-one markers displayed significant associations with plant height (PH) and detected 15 QTLs on all barley seven chromosome (Table 11).The range of *P* values was from 9.64E-04 to 2.53E-05 and the R^2 4.10% (*PH.2H-148.16*). Except for the *PH-3H-59-62*, all QTLs were specific to single environments.

Table 11: Summary of QTL associated with plant height (PH)

QTL	SNP	Chr	cM	*P*-value	R^2	Env.	Reference gene
PH.1H-126.23	11_10911	1	126.23	3.48E-04	3.91	JS15	
PH.2H-18.91	SCRI_RS_196192	2	18.91	2.70E-04	4.08	SE16	
PH.2H-74.15	12_11274	2	74.15	4.41E-04	3.79	SE16	*sdw3*
PH.2H-148.16	SCRI_RS_206020	2	148.16	2.53E-04	4.10	JS15	
PH.3H-8.43	11_21398	3	8.43	6.44E-04	3.55	JS15	
PH-3H-59-62	12_31011	3	59.56	4.41E-04	3.79	SE16	*qPLH-1*
	SCRI_RS_225522	3	62.39	5.56E-04	3.65	MCH15	
	11_10350	3	62.54	6.51E-04	3.58	MCH15	
PH.3H-98.37	12_30375	3	98.37	5.84E-04	3.61	JS15	Zhou et al. 2015
PH.3H-132.93	SCRI_RS_175682	3	132.93	9.64E-04	3.34	AT16	*sdw1/denso*
PH.4H-85.84	SCRI_RS_233444	4	85.84	6.92E-04	3.57	AT16	Malosetti et al.2011
PH.4H-102.18	SCRI_RS_170388	4	102.18	4.11E-04	3.82	MCH15	Malosetti et al.2011
PH.5H-43	SCRI_RS_236777	5	43.68	3.13E-04	3.98	MCH15	
	11_10177	5	43.96	2.91E-04	4.05	MCH15	
PH.5H-80.35	SCRI_RS_220136	5	80.35	6.47E-04	3.58	MCH15	
PH.5H-95	11_11361	5	95	3.47E-04	3.95	MCH15	
PH.5H-139.1	12_31165	5	139.1	4.83E-04	3.75	AT16	Malosetti et al.2011
PH.6H-68.2	11_11349	6	68.2	6.44E-04	3.55	JS15	

2.3. Spike Length (SL)

In total, 37 SNP markers were significantly associated with spike length (SL), yielding in 22 unique QTLs; the highest among the studied agronomic traits. Highly significant association with $-\log10_P = 4.54$was from chromosome 5H at 60.40 cM (*SL.5H-60-62*) and explained the maximum phenotypic variation which was 5.43% (Table 12). Ten QTLs had more than one significant SNP marker, out of which, eight QTLs with significant SNPs across different environments.

Table 12: Summary of QTL associated with spike length (SL)

QTL	SNP	Chr	cM	*P*-value	R^2	Env.	Reference QTL
SL.2H-54.25	11_20602	2	54.25	7.14E-04	3.52	AT16	
SL.2H-91.01	12_11285	2	91.01	6.87E-04	3.55	JS15	
	12_11285	2	91.01	1.47E-04	4.43	MCH15	
SL.2H-141.78	SCRI_RS_109266	2	141.78	4.72E-04	3.75	MCH15	*Slc.2.2*
SL.3H-17.49	SCRI_RS_151808	3	17.49	4.06E-04	3.85	AT16	
SL.3H-49-52	12_11387	3	49.29	1.30E-04	4.50	MCH15	*qSL-2*
	12_30009	3	51.77	3.26E-04	3.96	MCH15	
	12_31502	3	52.76	4.20E-04	3.82	SE16	
SL.3H-90.23	SCRI_RS_158070	3	90.23	7.62E-04	3.47	MCH15	*Slw.2.3*
	SCRI_RS_80331	3	90.23	7.62E-04	3.47	MCH15	
SL.4H-0.78	SCRI_RS_149873	4	0.78	1.10E-04	4.61	SE16	
SL.4H-28	12_10562	4	28.89	6.87E-04	3.55	JS15	
	12_10562	4	28.89	1.47E-04	4.43	MCH15	
SL.4H-51	SCRI_RS_143191	4	51.4	4.85E-04	3.75	AT16	
	SCRI_RS_9618	4	51.4	4.85E-04	3.75	AT16	
SL.4H-59.52	12_30455	4	59.52	1.08E-04	4.61	MCH15	
SL.4H-99.72	11_20454	4	99.72	7.78E-04	3.47	AT16	
SL.4H-112	11_10387	4	111.97	3.26E-04	3.98	AT16	*Slc.2.3*
SL.5H-45	SCRI_RS_224213	5	44.93	3.29E-04	3.98	AT16	
	12_30011	5	44.99	5.76E-04	3.76	MCH15	
SL.5H-60-62	11_20096	5	60.49	2.88E-05	5.43	AT16	
	12_21128	5	62.49	1.15E-04	4.58	MCH15	
SL.5H-97	12_30056	5	97.29	6.87E-04	3.55	JS15	*qSL-4*
	12_30056	5	97.29	1.47E-04	4.43	MCH15	
SL.5H-166.81	SCRI_RS_223728	5	166.81	1.43E-04	4.47	AT16	
SL.6H-55.03	12_30120	6	55.03	4.25E-05	5.17	MCH15	
SL.6H-78.4	11_20654	6	78.4	1.97E-04	4.34	MCH15	*qSL-5*
SL.6H-100.85	SCRI_RS_161117	6	100.85	4.06E-04	3.85	AT16	
SL.7H-68-70	12_11492	7	67.92	2.98E-04	4.02	MCH15	
	12_30481	7	70.54	3.48E-04	3.92	MCH15	
	SCRI_RS_102957	7	70.61	1.04E-04	4.66	AT16	
	11_10773	7	70.61	1.42E-04	4.47	AT16	
	11_10394	7	70.68	6.14E-04	3.61	AT16	
SL.7H-126-130	SCRI_RS_126437	7	126.27	3.86E-04	3.87	SE16	
	11_20139	7	128.26	1.42E-04	4.47	AT16	
	SCRI_RS_197886	7	130.03	5.66E-04	3.67	AT16	
SL.7H-140.86	12_30826	7	140.86	1.37E-04	4.47	MCH15	

2.4. Grain Yield (GY)

One of the most complex and important agronomic traits is grain yield. Across the chromosomes 15 QTLs were significantly associated with GY (Table 13). The highly significant SNP marker SCRI_RS_225522 (62.39 cM) with a *P* value of 5.88E-05 explained the highest phenotypic variation of 5.01%. The same marker was significantly associated with GY in MCH15 and AT16, making *GY.3H-62-64* the only multi-environment QTL associated with grain yield.

Table 13: Summary of QTL associated with grain yield (GY)

QTL	SNP	Chr	cM	*P*-value	R^2	Env.	Reference
GY.1H-47.95	12_11107	1	47.95	3.70E-04	3.87	MCH15	Mansour et al. 2013
GY.2H-3.15	SCRI_RS_211923	2	3.15	2.24E-04	4.18	AT16	
GY.2H-7-11	SCRI_RS_151082	2	7.44	3.30E-04	4.21	SE16	
	SCRI_RS_209516	2	11.4	6.60E-04	3.61	AT16	
GY.2H-135.76	SCRI_RS_149592	2	135.76	3.41E-04	3.93	AT16	Matthies et al. 2014
	SCRI_RS_175074	2	135.76	3.41E-04	3.93	AT16	
GY.3H-62-64	SCRI_RS_225522	3	62.39	4.01E-04	3.92	AT16	
	SCRI_RS_225522	3	62.39	5.88E-05	5.01	MCH15	
	SCRI_RS_160216	3	64.16	6.98E-04	3.51	AT16	
GY.4H-88.84	11_20732	4	88.84	6.63E-04	3.86	SE16	Matthies et al. 2014
GY.4H-111.97	SCRI_RS_85607	4	111.97	7.43E-04	3.48	JS15	
GY.5H-91.16	SCRI_RS_169596	5	91.16	1.84E-04	4.38	AT16	
GY.5H-125.76	11_21241	5	125.76	5.51E-04	4.21	MCH15	*vrn-H1*
GY.5H-139.24	11_21355	5	139.24	9.10E-04	3.65	SE16	Matthies et al. 2014
GY.5H-155.56	SCRI_RS_160183	5	155.56	7.41E-04	3.51	JS15	Matthies et al. 2014
GY.5H-167.71	12_31210	5	167.71	1.76E-04	4.31	JS15	boudiar et al. 2016
GY.6H-59-63	SCRI_RS_108698	6	58.91	1.05E-04	4.62	JS15	
GY.6H-63.46	SCRI_RS_152841	6	63.46	3.35E-04	3.94	JS15	Matthies et al. 2014
GY.6H-113.24	SCRI_RS_111434	6	113.24	1.88E-04	4.28	AT16	

2.5. Thousand Kernel Weight (TKW)

A total of 28 SNP markers have been found to be significantly associated with thousand kernel weight trait, making 19 QTLs after the cutoff 3.6 cM LD decay (Table 14). *P* values ranged

from 2.03E-06 to 9.58E-04 and the phenotypic variation explained by these SNPs was up to 7.05% (*TKW.6H-91-92*). Six QTLs had more than one significant SNP marker, three of them were significant in more than one environment. The most consistent QTL, *TKW.5H-42-43* had four highly significant SNP at AT16 and SE16 and explained up to 6.89% of the phenotypic variation (Table 14).

Table 14: Summary of QTL associated with 1000-kernel weight (TKW)

QTL	SNP	Chr	cM	*P*-value	R^2	Env.	Reference QTL
TKW.1H-18.27	SCRI_RS_152464	1	18.27	7.60E-05	4.84	AT16	
TKW.1H-132.58	12_30934	1	132.58	8.92E-04	3.35	SE16	
TKW.2H-6.59	SCRI_RS_133377	2	6.59	3.69E-04	3.92	AT16	
TKW.2H-58-59	11_20585	2	58.64	8.95E-04	3.35	SE16	Comadran et al. 2011
	SCRI_RS_173145	2	59.07	2.72E-04	4.10	AT16	
TKW.2H-70.82	11_11435	2	70.82	3.79E-04	3.89	AT16	Teulat et al. 2002
TKW.3H-51.63	SCRI_RS_11126	3	51.63	9.94E-05	4.68	AT16	*QTL9_TGW*
TKW.4H-51.13	SCRI_RS_188822	4	51.13	6.30E-04	3.61	AT16	
TKW.4H-78-81	SCRI_RS_171874	4	78.47	3.41E-05	5.32	AT16	
	11_20513	4	81.57	9.58E-04	3.25	MCH15	
TKW.4H-97-98	SCRI_RS_169580	4	97.66	7.22E-04	3.51	AT16	
	SCRI_RS_139508	4	98.94	1.14E-04	4.60	AT16	Comadran et al. 2011
TKW.4H-103.9	SCRI_RS_201871	4	103.9	7.31E-05	5.05	AT16	
TKW.5H-42-43	SCRI_RS_150377	5	42.15	2.99E-04	4.07	AT16	*Vrs2*
	12_31492	5	42.85	1.35E-04	4.52	AT16	
	SCRI_RS_181514	5	43.68	2.64E-06	6.89	AT16	
	SCRI_RS_237910	5	43.68	7.28E-04	3.47	SE16	
	SCRI_RS_167829	5	43.76	9.29E-05	4.72	AT16	
TKW.5H-129.44	SCRI_RS_171042	5	129.44	5.67E-06	6.41	AT16	
TKW.6H-73.8	SCRI_RS_206976	6	73.8	5.83E-05	5.07	AT16	*qTKW*
TKW.6H-91-92	SCRI_RS_131929	6	91.71	2.03E-06	7.05	AT16	
	SCRI_RS_152675	6	92.28	2.38E-06	6.95	AT16	
TKW.6H-97.38	SCRI_RS_234362	6	97.38	4.04E-04	3.85	AT16	
TKW.6H-118.41	SCRI_RS_174459	6	118.41	5.88E-05	4.99	AT16	
TKW.7H-67.92	SCRI_RS_108830	7	67.92	1.34E-04	4.39	MCH15	
TKW.7H-70	SCRI_RS_200107	7	70.54	2.58E-05	5.49	AT16	
	SCRI_RS_133777	7	70.61	7.24E-04	3.51	AT16	
TKW.7H-92.07	SCRI_RS_174699	7	92.07	4.02E-04	3.86	AT16	

3. Discussion

Studies aiming at the identification of QTL for agronomic traits (agro-morphological, yield and yield components) in barley are quite abundant in literature. However, given the quantitative nature of these traits and being highly affected by a large QTL x environment effect make it difficult to find stable QTLs across environments (Romagosa et al. 1999). For this reason, it is important to constantly identify QTLs for potential application in targeted environments or closely related environments to be used in the barley breeding programs. In the present chapter we describe the application of genome-wide association studies in a panel of agronomically diverse spring barley. For each of the analyzed trait, we identified 15 to 22 QTLs. Most of the QTLs detected under this study were not consistent across locations and were environment-specific, although significant cross-over interactions were detected for few of them. Some of the QTLs have been identified in previous studies (Tables 10 to 14) but a substantial portion of QTL was not reported previously, therefore these QTL are considered as novel.

Days to heading (DH) reflects the adaptation of a plant to its environment and is a complex trait effected by numerous QTL both in outbreeding and in inbreeding species. Many SNP markers were found to be significantly associated with DH in our study (Table 10) and we report a total of 23 significant SNPs defining 17 QTLs. Some of these QTLs fall within or close to genomic regions that were previously reported to harbor major genes and minor genes including *Ppd-H1*, *eam6, vrs3, HvFT4, HvGI* and *HvCO3* (Table 10). The genes *Ppd-H1, eps2* are considered as major photoperiodic and heading date genes and were fist described by Laurie et al. (1994) from a double-haploid mapping population of a cross derived from the winter barley variety ‘’Igri’’ and the spring variety ‘’Triumph’’. In barley, *Ppd-H1* and *Ppd-H2* genes are the principal determinants of flowering time under long and short photoperiods, respectively (Karsai et al. 1997). Since all our experiment consisted of spring barley cultivated in long photoperiod locations, it is not surprising to map *Ppd-H1* gene only. Further, the significant SNP markers BK_12 and BK_16 in the iSelect were derived from the *Ppd-H1* gene on chromosome 2H. The gene *eam6* in the centromeric region of chromosome 2H, formally *eps2* (earliness *per se* locus) is described as major gene controlling heading date and was also detected in our association analysis. Further, Boudiar et al. (2016) identified a QTL associated with flowering time in a double-haploid population derived from the Spanish barley backcross (SBCC073 x Orria) x Orria at 56.75 cM on chromosome 3H. The QTL *DH.3H-51-53* is in 3 cM distance from the one mapped by Boudiar et al. (2016) suggesting that the two might be

the same. Similarly *DH.2H-82.08* is likely to be same as *flo-2H-1,* a flowering time associated QTL detected by a study of Peighambari et al. (2005) at 80 cM on chromosome 3H . In addition to the known major and minor genes involved in the days to heading/flowering time in barley, we identified eight novel QTL associated with this trait. These findings emphasizes that flowering time in barley is under complex genetic control and can be explained by many small effect of flowering time QTL.

Plant height is considered one of the most important traits in barley and is under polygenic control (Wang et al. 2014; Zhou et al. 2015). Since the green revolution causing genes in wheat and rice were identified, the use of semi-dwarf genes has greatly improved yields through loss reduction arising from lodging and the increase of harvest index. In this study, we detected highly significant marker-trait associations for PH located on all chromosomes except 7H (Table 11). Seven out of the fifteen mapped QTLs are consistent with major and minor genes described in earlier studies (Table 11). For instance The QTL *PH.2H-74.15* on chromosome 2H coincides with the mapping position of *sdw3* which plays a major role in gibberellins-insensitive dwarfing barley (Gottwald et al. 2004) and *PH.3H-132.93* mapped on the long arm of chromosome 3H is approximately 5 cM away from the two allelic form of the dwarfing gene *denso/sdw1* (Jia et al. 2011). Further, the QTL *PH.3H-98.37* might be the same as the major QTL mapped at 105.5 cM on chromosome 3H, found associated with plant height by Zhou et al. (2015) and explained 44.5% of phenotypic variation. Similarly, four QTLs identified in this association analysis have been previously detected from studies undertaken by Malosetti et al. 2011 and Shamasbi et al. 2017 (Table 11). The remaining eight QTLs are considered novel and might be useful for breeding dwarf or semi-dwarf barley cultivars in the future.

The largest number of QTLs (22) detected in GWAS for agronomic traits was associated with spike length, of which seven have already been reported (Table 12). Xue et al. (2010) mapped three QTLs on chromosome 2H, 3H and 4H (*Slc.2.2*; *Slw.2.3*; and *Slc.2.3*) from a Yerong x Franklin double haploid population, which corresponds to *SL.2H-141.78*, *SL.3H-90.23* and *SL.4H-112*, respectively. Similarly, we also identified three QTLs hitting genomic regions mapped in a study done by Shamasbi et al. (2010), using a DH population derived from a cross between the Australian cultivar 2-rowed Clipper and the Algerian 6-rowed landrace Sahara 3771, namely *qSL-2*, *qSL-4* and *qSL-5*, on chromosome 2H, 4H and 5H, respectively (Table 12). The QTLs *SL.3H-49-52* and *SL.6H-55.03* are in ~2cM distance from the flanking SSR markers Bmac0067 (3H) and GBM1027 (6H), associated with spike length in an advanced

backcross BC_2F_8 population (Gyenis et al. 2007). The remaining 15 QTLs were not previously reported in earlier studies, and therefore are considered novel.

Grain yield is one of the most important but complex trait in cereals breeding. In this section we report the identification of fifteen QTLs associated with GY in barley on all chromosomes except 7H (Table 13). The *GY.1H-47.95* QTL is in ~2cM distance from the SNP marker 11_10275, significantly associated with GY in subset 112 recombinant inbred lines (RILs) phenotypic in multi-environments in the Mediterranean region (Mansour et al. 2013). Most of the associated genomic regions for grain yield were also significant in GWAS of Matthies et al. (2014) similar to the QTL *GY.2H-135.76*, *GY.4H-88.84*, *GY.5H-139.24*, *GY.5H-155.56* or *GY.6H-63.46* reported in this study (Table 11). Furthermore, *GY.5H-167.71* falls within the confidence interval of the grain yield QTL mapped by Boudiar et al. (2016) on chromosome 5H contributed by a Mediterranean landrace from Spain. This findings are not surprising since the majority of the germplasm used in this association mapping panel is also from the Mediterranean region and may have shared same alleles. The vernalization gene *vrn-H1* was co-located with QTL on chromosome 5H for grain yield (*GY.5H-125.76*). In barley, vernalization is determined by three major loci *vrn-H1, vrn-H2* and *vrn-H3*. Winter-type barley is considered vernalization-responsive which involves the haplotype *vrn-H1vrn-H2vrn-H3* whereas all other allelic combinations at three loci will confer a spring-type barley. Wang et al. (2010) reported an effect of *vrn-H1* on grain yield in an advanced backcross study of a *Hordeum spontaneum* x elite spring barley population. On the other hand, Comadran et al. (2011) found significant QTL environment interaction for SNPs closely linked to *vrn-H1* and *vrn-H2* in a genome-wide association study of yield for a diverse panel of barley genotypes, phenotyped over a number of different Mediterranean environments. Our results are consistent with these previous findings and strongly highlights the involvement of *vrn-H1* in grain yield and yield components.

The grain weight, usually expressed as thousand kernel weight (TKW) is one of the most important yield component in barley. A total of 28 highly significant marker-trait associations were detected for TKW resulting in 19 QTLs (Table 14). Comadran et al. (2011) identified two marker-trait associations at 62.82 cM on 2H and 92.38 cM on 4H which might be same as *TKW.2H-58-59* and *TKW.4H-97-98* identified in this study. Similarly the flanking SSR marker EBmac0684 associated with TKW is likely same as *TKW.2H-70.82* (Teulat et al. 2002). This is not surprising since the barley RILs used is Teulat et al. (2002) is derived from two ICARDA

accessions (Arabi Aswad and Er/Apm) that can be found in the parentage of several genotypes used in this study and therefore detecting the same QTL is expected. Furthermore, *TKW.3H-51.63* and *TKW.6H-73.8* were also reported in earlier studies by Pasam et al. (2002) and Shamasbi et al. (2017), respectively. The consistent QTL *TKW.5H-42-43* with five co-segregating SNPs (up to $-\log_{10}(P) = 5.58$ and $R^2 = 6.89\%$) co-located with *Vrs2*, one of the five independent genes reported to control the six-rowed spike phenotype (Lundqvist et al. 1997). It is known that most two-rowed barley varieties have large grains than six-rowed varieties and detecting the six-row phenotype gene *Vrs2* highlights the negative effect of six-row genotypes on grain weight in the association mapping analysis. The thirteen novel QTL identified in this study maybe useful for MAS for TKW, especially the highly significant markers, which explained up to 7.05 % of the phenotypic variation (e.g. SCRI_RS_131929, SCRI_RS_152675).

Conclusion

We have reported several known and novel QTLs associated with five key traits in barley improvement. Most of them were not consistent across locations as they were detected as interactions with the environment, although some cross-over interactions were detected for few of them. This is not uncommon for quantitative traits as they are highly influenced by the environment and suggests that the breeding strategy should be environment targeted (or similar environments) by the deployment of small effect, environment-specific QTLs in elite cultivars along with large effect QTLs.

VII. Conclusions and outlook

The use of Genome-Wide Association Studies (GWAS) has significant impacts on the discovery of Quantitative Trait Loci (QTL). The multiple advantages of association mapping over traditional bi-parental mapping (e.g. high resolution mapping, use of natural and diverse populations) has made it cost- and time-effective over traditional bi-parental mapping. However, the extent of linkage disequilibrium in the association mapping panel plays a crucial role in detecting significant variant of the phenotype of interest at moderate to high resolution. And despite the advantages of GWAS to pinpoint genetic polymorphisms underlying traits of interest, this approach may result in detecting false positives due to population structure. Several statistical models have been widely used to correct the effect of population structure on marker-trait associations.

We used one of the most stringent model, the mixed linear model (MLM), in this study accounting for population structure and relatedness of genotypes followed by correcting for false discovery rate (FDR) to reduce the risk of incurring false positives and spurious associations. Seedling and adult-plant NFNB and SFNB resistance was recorded as well as the scoring of five important agronomic traits in a population of 336 spring barleys led to the detection of significant marker-trait associations using GWAS. Co-localization of multiple QTL with major and minor genes that are reported in previous reports validated the approach and indicated the pleiotropic effects of these associations.

The discovery of segregating significant associations, specific to different environments, reflected the quantitatively inherited nature of the studied traits and the allelic diversity at these genomic regions in the association mapping panel AM-2014. In most cases, several significant marker-trait associations were observed over all seven barley chromosomes suggesting that the studied traits are under the control of several QTL with minor and/or major effect. The relatively observed low LD has a great potential and can be exploited in candidate gene discovery and the use of the 9K iSelect SNP markers (7842 SNPs on the array) was demonstrated as sufficient in this study in terms of marker coverage, the detection and the increase of highly significant marker-trait associations at genome-wide level. Marker

information and the detailed phenotype data generated for cultivars in this study will be a useful information for marker-assisted and genomic selection as well as haplotype-based association analysis in barley breeding.

Overall, results from the present study provide an insight into the genetic architecture of resistance to important barley diseases (NFNB and SFNB) and agronomic traits for barley (DH, PH, SL, GY, TKW). In total, we identified 140 QTL for these traits. Some genomic regions harbor QTL for more than one trait, 58 QTL (out of 140) have been found to concur with previously mapped QTL. For all traits together, 82 novel QTL have been detected.

Studies at the genome-wide level uncovered several environment-specific associations that provide the gateway to the efficient use of genomic resources for breeding that were not tapped yet. In the future, associations discovered in this study should be validated before marker information is incorporated in selection decisions, or investment in identification of causal factors and gene cloning. The results in this study can be validated by replicating the experiments or through comparisons in different populations. The probability of observing false positives in this case becomes small in significant associations are confirmed in validation populations. Another way is the use of carefully generated bi-parental mapping populations. Then the underlined candidate gene(s) can be characterized using mutants and transgenic approaches.

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Supplemental material

Table S1a: Analysis of variance of agronomic traits measured in Marchouch and Jemaa Shaim, Morocco in 2014-2015.

Sources of variation	df	[a]DH	PH	SL	NFNB	SFNB	G/S	BY	GY	HI
Genotype (G)	335	72.4**	217.6**	4.151**	2366.4**	2707.4**	781.5**	3.456**	0.76674**	0.0070**
Environment (E)	1	48681.5**	357290.3**	83.560**	80272.8**	2443.6^{ns}	16.0^{ns}	15363.6**	3342.8**	4.249**
G×E	334	61.8**	60.6^{ns}	2.340**	1025.5**	1409.9**	228.3^{ns}	2.596^{ns}	0.6446**	0.0051^{ns}
Error	649	2.6	82.2	1.52	812.2	781.0	201.7	2.66843	0.493	0.005

[a]PH-Plant height, SL-Spike length, NFNB-Net form of net blotch, SFNB-Spot form of net blotch, G/S-Grain per spike, BY-Biomass yield, GY-Grain yield, HI-Harvest index

*, ** are significant at 0.05 and 0.01 probability levels

Table S1b: Analysis of variance of agronomic traits measured either in Marchouch or Jemaa Shaim, Morocco in 2014-2015.

Source of Variation	df	[a] DM	TKW	HW	PM-Adult	[b]PM-Seedling
Replication (R)	1	181.2**	120.9**	4.34**	0.0554	2.93*
Genotype (G)	335	47.8**	45.7**	67.71**	1292.6**	0.614^{ns}
Error	335	20.1	9.15412	0.936	0.0276	0.6555688

[a] DM-Days to maturity,TKW-1000 kernel weight, HW-Hectoliter (test) weight,PM-Adult-Powdery mildew severity recorded at adult stage using double digit in Marchouch,

[b]PM-Seedling-Powdery mildew recorded at seedling stage using 1-5 scale in Jemaa Shaim where 1 is resistance response and 5 is susceptible

*, ** are significant at 0.05 and 0.01 probability levels

Table S2: Summary of QTL associated with Net Form of Net Blotch (NFNB) resistance at the seedling and adult plant stages

QTL	SNP ID	Ch [a]	Position [b] (cM)	*P*-value [c]	R^2 (%)	SNP	MAF [d]	Additive effect [e]	Env./ Isolate	Ref [f]
QRptta-1H-4.11	SCRI_RS_161137	1H	4.11	8.38E-04	3.42	T/C	C(0.34)	T(-2.47)	SE.15	
QRptts-1H-92-93	SCRI_RS_194371	1H	92.35	9.20E-04	3.4	T/C	T(0.33)	C(-0.01)	Ptt.19	
	11_10522	1H	93.7	3.74E-04*	3.9	A/G	A(0.12)	G(-1.21)	Ptt.19	
QRptta-1H-125.99	SCRI_RS_165886	1H	125.99	9.05E-04	3.39	A/G	G(0.36)	G(4.25)	JS.15	
QRptts-2H-7.44	SCRI_RS_103515	2H	7.44	5.60E-04*	3.6	T/C	C(0.36)	C(0.66)	TD10	1
QRptta-2H-40.79	12_20326	2H	40.79	5.51E-06*	6.41	A/G	A(0.05)	G(-8.60)	SE.15	2
QRptta-2H-57-59	12_10948	2H	57.15	3.10E-04*	4.00	A/G	A(0.07)	G(-7.77)	MCH.15	3
	11_20442	2H	59.35	9.32E-04	4.08	A/G	G(0.35)	G(5.45)	IAV.15	
QRptta-2H-92.21	11_20498	2H	92.21	1.45E-04*	5.43	A/G	G(0.40)	A(5.72)	IAV.15	
QRptta-2H-114-117	SCRI_RS_162820	2H	114.31	7.35E-04*	3.57	A/C	A(0.33)	C(0.77)	IAV.16	
QRptta-2H-126.77	SCRI_RS_201870	2H	117.39	3.04E-04*	4.09	T/C	C(0.35)	T(-1.90)	IAV.16	
	SCRI_RS_155734	2H	126.77	4.53E-04*	3.78	T/C	T(0.31)	C(-4.41)	MCH.15	
	SCRI_RS_121952	2H	126.77	5.43E-04*	3.67	T/C	C(0.30)	T(-4.37)	MCH.15	
QRptt.2H-132.15	12_31461	2H	132.15	5.16E-04*	3.70	A/G	A(0.14)	A(3.34)	SE.15	
	11_10625	2H	132.15	5.13E-04*	3.7	A/G	G(0.34)	G(-0.69)	TD10	
QRptta-2H-143.13	SCRI_RS_159526	2H	143.13	7.59E-04	3.48	A/C	A(0.27)	C(8.43)	AT.16	3
	SCRI_RS_159526	2H	143.13	6.82E-04*	3.55	A/C	A(0.27)	C(4.12)	MCH.15	
	SCRI_RS_159526	2H	143.13	9.20E-04	3.37	A/C	A(0.27)	C(10.10)	SE.16	
	BOPA1_678-310	2H	729751165-729751166	7.65E-04	3.48	A/G	A(0.30)	G(-4.23)	MCH.15	
	BOPA2_12_10689	2H	669122451-669122451	6.13E-04*	3.61	A/G	G(0.17)	A(3.42)	SE.15	
QRptta-3H-118.30	SCRI_RS_219896	3H	118.30	4.54E-04*	3.86	T/C	T(0.08)	C(-3.02)	IAV.16	4
QRptta-3H-144.65	12_20369	3H	144.65	5.77E-04*	3.64	A/C	C(0.11)	A(-5.91)	MCH.15	
QRptta-3H-154-155	12_10215	3H	154.15	1.72E-05**	7.01	A/G	A(0.07)	G(-10.81)	IAV.15	
	12_10215	3H	154.15	7.95E-04	3.45	A/G	A(0.09)	G(-6.59)	JS.15	

QTL	SNP ID	Ch [a]	Position [b] (cM)	*P*-value [c]	R^2 (%)	SNP	MAF [d]	Additive effect [e]	Env./ Isolate [f]	Ref [g]
	SCRI_RS_208722	3H	155.03	8.94E-04	3.37	T/C	T(0.32)	T(-4.42)	JS.15	
QRptta-4H-81.57	12_10022	4H	81.57	5.77E-04*	4.42	A/G	A(0.36)	A(5.30)	IAV.15	
QRptts-4H-97.66	SCRI_RS_193252	4H	97.66	8.57E-04	3.5	A/G	A(0.21)	G(-0.92)	Ptt.19	
	SCRI_RS_153184	4H	97.66	8.57E-04	3.5	T/G	T(0.21)	G(-0.92)	Ptt.19	
QRptta-5H-43.76	12_20770	5H	43.76	8.64E-04	3.51	C/G	C(0.21)	G(-2.12)	IAV.16	
QRptts-5H-80.35	12_31271	5H	80.35	5.38E-04*	3.7	C/G	C(0.07)	G(-1.42)	Ptt.19	
QRptts-5H-130.03	SCRI_RS_7933	5H	130.03	5.03E-04*	3.8	T/C	C(0.30)	C(0.92)	Ptt.19	5, 6
QRptta-5H-139.38	12_10016	5H	139.38	8.39E-04*	3.42	A/G	A(0.20)	G(-4.61)	MCH.15	
QRptta-5H-143.4	SCRI_RS_173583	5H	143.4	7.29E-04	3.52	A/G	G(0.44)	G(-4.18)	JS.15	
QRptta-5H-160.49	SCRI_RS_195241	5H	160.49	9.50E-04	3.35	T/C	T(0.35)	C(-7.86)	AT.16	
	SCRI_RS_195241	5H	160.49	8.64E-04	3.41	T/C	C(0.35)	T(-9.67)	SE.16	
QRptta-6H-35.62	11_10994	6H	35.62	9.13E-04	3.44	A/G	G(0.47)	A(-1.72)	IAV.16	
QRptta-6H-49.79	12_10199	6H	49.79	2.68E-04*	4.17	A/G	A(0.13)	G(-2.66)	IAV.16	7
QRptt.6H-54-55	SCRI_RS_140091	6H	54.89	4.48E-04*	3.81	A/C	C(0.31)	A(9.94)	AT.16	1, 2,
	SCRI_RS_140091	6H	54.89	5.11E-04*	3.74	A/C	C(0.31)	A(11.89)	SE.16	7, 8,
	SCRI_RS_146867	6H	54.89	5.66E-05**	5.1	T/C	C(0.39)	T(0.98)	Ptt.19	9, 10
	SCRI_RS_114613	6H	55.38	6.57E-04*	3.63	T/C	C(0.16)	T(-2.25)	IAV.16	
QRptts-6H-78.4	SCRI_RS_1937	6H	78.4	7.75E-04	3.5	A/C	A(0.37)	C(0.77)	Ptt.19	4
QRptts-6H-98.55	SCRI_RS_155564	6H	98.55	6.94E-04*	3.6	T/C	T(0.45)	C(0.80)	Ptt.19	11
QRptta-7H-0.39	SCRI_RS_7933	7H	0.39	8.03E-04	3.43	A/C	A(0.38)	A(-4.16)	JS.15	
QRptta-7H-23.02	SCRI_RS_169269	7H	23.02	2.31E-04*	4.26	T/C	T(0.36)	C(-1.93)	IAV.16	
QRptta-7H-42.28	11_21528	7H	42.28	6.44E-04*	3.57	A/T	A(0.19)	T(-2.92)	SE.15	
QRptts-7H-74.29	SCRI_RS_115426	7H	74.29	4.93E-04*	3.8	A/G	A(0.41)	G(0.90)	Ptt.19	

[a] Chromosome; [b] Genetic position of SNPs anchored using POPSEQ barley (Maescher et al. 2013), some markers with unknown genetic position was anchored using physical map of Morex genome sequence; [c] *, ** *P*-value corrected using FDR at 0.05 and 0.01 probability levels, respectively; [d] Minor allele frequency; [e] Effect contributed by the respective marker on a 1-10 scale for seedlings and 0-100% severity for adult plants; [f] Environment and cropping season or the isolate used for NFNB screening; [g] Ref= Previously published NFNB resistance QTL associated with SNPs identified in this study:

[1] Wonneberger et al. 2017a, [2] Steffenson et al. 1996, [3] Cakir et al. 2011, [4] Grewal et al. 2008, [5] Knig et al. 2013, [6] Wonneberger et al. 2017b, [7] Richards et al. 2017, [8] Friesen et al. 2006, [9] St. Pierre et al. 2009, [10] Koladia et al. 2016, [11] Abu Qamar et al. (2008)

Table S3: Blast search for significant SNP sequences associated with NFNB resistance

SNP ID	**Gene annotation using barley map by Cantalapiedra et al. (2015)**	***E* Value**	**Gene annotation using BLAST (NCBI)**	**LOC/GI**
SCRI_RS_161137	NBS-LRR-like protein, Receptor like protein kinase	2.00E-37	*Aegilops tauschii* chromosome 1Ds prolamin gene locus	LOC109745987
11_10522	Tubby-like F-box protein 9	4.00E-83	*Aegilops tauschii* subsp. *tauschii* tubby-like F-box protein 9	LOC109767169
SCRI_RS_103515	Cullin-associated NEDD8-dissociated protein	2.00E-47	*Aegilops tauschii* subsp. *tauschii* cullin-associated NEDD8-dissociated protein 1-like	LOC109770968
12_20326	Xylose isomerase	3.00E-51	*H.vulgare* xylose isomerase gene	
12_10948	Aquaporin, heat shock transcription factor A3	2.00E-53	*H.vulgare* aquaporin (pip2;6)	
11_20442	NAC domain-containing protein 78, Zinc finger protein	2.00E-105	*Aegilops tauschii* subsp. *tauschii* L-ascorbate peroxidase 7, chloroplastic	LOC109747802
SCRI_RS_162820	Yellow stripe-like transporter 12, Beta-fructofuranosidase	2.00E-33	*Aegilops tauschii* subsp. *tauschii* beta-fructofuranosidase, insoluble isoenzyme 7-like	LOC109750703
SCRI_RS_121952	LRR receptor-like protein kinase, Filament-like plant protein	8.00E-42	*Aegilops tauschii* subsp. *tauschii* filament-like plant protein 7	LOC109746920
SCRI_RS_219896	Kelch-like protein 14	1.00E-19	*Aegilops tauschii* subsp. *tauschii* F-box/kelch-repeat protein	LOC109738242
12_20369	LRR receptor-like protein kinase, Aldehyde dehydrogenase	6.00E-43	*Brachypodium distachyon* obg-like ATPase 1	LOC100828812
12_10215	Disease resistance-responsive (Dirigent-like protein) family protein	3.00E-41	*Aegilops tauschii* subsp. *tauschii* glutathione S-transferase 1	LOC109776880
12_10215	Disease resistance-responsive (Dirigent-like protein) family protein	3.00E-41	*Aegilops tauschii* subsp. *tauschii* glutathione S-transferase 1	LOC109776880
12_10022	Copper-transporting ATPase 2,	2.00E-38	*Aegilops tauschii* subsp. *tauschii* copper-transporting ATPase PAA2	LOC109772413
SCRI_RS_193252	BHLH transcription factor	6.00E-23	*Aegilops tauschii* subsp. *tauschii* transcription factor bHLH93-like	LOC109735449
12_20770	GDP-L-galactose phosphorylase, Pectinesterase	4.00E-45	*Aegilops tauschii* subsp. *tauschii* GDP-L-galactose phosphorylase 1-like	LOC109736377

SNP ID	**Gene annotation using barley map by Cantalapiedra et al. (2015)**	***E* Value**	**Gene annotation using BLAST**	**LOC/GI**
12_31271	NB-ARC domain-containing disease resistance protein	2.00E-52	*Hordeum vulgare* NAC transcription factor	NAC048 gene
12_10016	30S ribosomal protein S19	3.00E-41	*Aegilops tauschii* subsp. *tauschii* 40S ribosomal protein S15-like	LOC109741611,
SCRI_RS_195241	Kinase family protein	5.00E-34	*Aegilops tauschii* subsp. *tauschii* shaggy-related protein kinase kappa-like	LOC109766472
SCRI_RS_195241	Kinase family protein	5.00E-34	*Aegilops tauschii* subsp. *tauschii* shaggy-related protein kinase kappa-like	LOC109766472
11_10994	LRR receptor-like protein kinase, NBS-LRR disease resistance protein family-1	2.00E-51	*Triticum aestivum* cyclophilin A-1 (CyP1) mRNA	-
12_10199	5'-AMP-activated protein kinase-like protein	1.00E-39	*Aegilops tauschii* subsp. *tauschii* eukaryotic translation initiation factor 5-like	(LOC109752314
SCRI_RS_146867	Disease resistance protein, Serine/threonine protein phosphatase 2A regulatory subunit B' subunit alpha	8.00E-42	*Zea mays* RmlC-like cupins superfamily protein	LOC103627425
SCRI_RS_114613	Serine carboxypeptidase (Carboxypeptidase D)	8.00E-22	*Aegilops tauschii* subsp. *tauschii* serine carboxypeptidase-like 34	LOC109746315
SCRI_RS_1937	PLANT CADMIUM RESISTANCE 2	6.00E-43	*Aegilops tauschii* subsp. *tauschii* cell number regulator 1-like	LOC109751059
SCRI_RS_155564	Ethylene responsive transcription factor 2a, GPI ethanolamine phosphate transferase	1.00E-39	*Aegilops tauschii* subsp. *tauschii* GPI ethanolamine phosphate transferase 2	LOC109760551
SCRI_RS_115426	HEAT repeat-containing protein, Aquaporin	2.00E-38	*Brachypodium distachyon* alcohol dehydrogenase	LOC100830644

Table S4: Summary of QTL associated with Spot Form of Net Blotch (SFNB) resistance at the seedling and adult plant stages

QTL	SNP_ID	CHR [a]	Position [b] (cM)	*P*-value [c]	R^2 (%)	MAF [d]	Additive effect [e]	Env./Isolate [f]
QRptma-1H-4.11	SCRI_RS_161137	1H	4.11	5.64E-04	3.62	0.34	-2.84	SE.16
QRptma-2H-40.79	12_20326	2H	40.79	6.82E-05	4.86	0.05	-8.45	SE.16
QRptma-2H-91-94	SCRI_RS_170162	2H	91.15	9.47E-04	3.41	0.20	-4.51	SE.15
	SCRI_RS_157097	2H	94.41	6.44E-04	3.64	0.07	-7.22	SE.15
QRptma-2H-107.37	11_10988	2H	107.37	9.92E-04	4.11	0.14	-1.05	IAV.16
QRptma-2H-132.15	12_31461	2H	132.15	1.99E-04	4.24	0.14	3.87	SE.16
QRptma-3H-50-51	11_11502	3H	50.71	3.79E-04	3.88	0.45	-2.16	AT.16
	11_10225	3H	51.63	4.92E-04	3.73	0.42	-1.83	AT.16
QRptma-3H-104.75	12_31269	3H	104.75	8.72E-04	3.37	0.18	3.85	SE.16
QRptma-3H-120-124	11_20527	3H	120.68	1.78E-04	4.32	0.15	-2.67	AT.16
	SCRI_RS_219896	3H	122.59	1.95E-04	4.27	0.08	-3.16	AT.16
	SCRI_RS_209963	3H	124.54	3.72E-04	3.89	0.08	-3.02	AT.16
QRptma-4H-24.48	SCRI_RS_143144	4H	27.48	3.67E-04	3.90	0.05	-3.57	AT.16
QRptms-4H-51.4	11_10261	4H	51.4	9.70E-04	3.54	0.27	-0.20	FGO
QRptma-4H-81.57	12_10022	4H	81.57	3.77E-04	3.85	0.39	2.86	SE.16
QRptma-5H-46-47	SCRI_RS_147429	5H	46.56	7.66E-04	3.54	0.29	-4.26	SE.15
	11_20306	5H	46.99	7.66E-04	3.54	0.29	-4.26	SE.15
	SCRI_RS_157305	5H	47.22	6.39E-04	3.64	0.29	-4.36	SE.15
	SCRI_RS_164068	5H	47.22	3.86E-04	3.96	0.28	0.67	SE.16
QRptma-5H-98.54	SCRI_RS_214241	5H	98.54	9.41E-04	3.36	0.45	-1.92	AT.16
QRptma-5H-143-144	SCRI_RS_153575	5H	143.68	1.54E-04	4.49	0.18	-5.56	SE.15
	11_10536	5H	144.51	4.36E-04	3.87	0.14	-5.52	SE.15
QRptms-5H-168.89	12_31481	5H	168.89	5.68E-04	3.82	0.18	0.26	FGO
QRptma-6H-2.62	SCRI_RS_199940	6H	2.62	8.74E-04	3.39	0.24	2.13	AT.16

QTL	SNP_ID	CHR [a]	Position [b] (cM)	*P*-value [c]	R^2 (%)	MAF [d]	Additive effect [e]	Env./Isolate [f]
QRptma-6H-65-67	11_10124	6H	65.93	5.00E-04	3.79	0.12	5.84	SE.15
	BOPA2_12_10348	6H	66.08	2.13E-05	5.70	0.10	7.52	SE.15
	11_11329	6H	67.92	2.75E-05	5.54	0.18	6.59	SE.15
QRptma-6H-86.97	SCRI_RS_8252	6H	86.97	5.18E-04	4.57	0.09	-1.34	IAV.16
QRptma-7H-3.82	12_31173	7H	3.82	1.82E-04	4.31	0.46	-1.84	AT.16
QRptma-7H-23.02	SCRI_RS_169269	7H	23.02	2.48E-05	5.50	0.37	-2.24	AT.16
	SCRI_RS_116905	7H	70.54	3.32E-04	4.89	0.43	0.90	IAV.15
QRptm-7H-70-71	SCRI_RS_126380	7H	70.54	6.03E-05	6.15	0.42	0.93	IAV.16
	SCRI_RS_235409	7H	70.54	1.26E-04	5.60	0.42	0.90	IAV.16
	SCRI_RS_139480	7H	70.54	1.63E-04	5.41	0.42	0.89	IAV.16
	SCRI_RS_185680	7H	70.54	5.18E-04	4.80	0.42	0.84	IAV.16
	SCRI_RS_152635	7H	70.57	1.32E-04	5.57	0.42	0.90	IAV.16
	12_30574	7H	70.61	9.43E-04	3.51	0.46	0.18	FGO
	12_10459	7H	70.61	3.32E-04	4.89	0.43	0.90	IAV.15
	SCRI_RS_219749	7H	70.61	1.90E-04	5.31	0.44	0.87	IAV.16
	12_10713	7H	70.61	3.32E-04	4.89	0.43	0.85	IAV.16
	12_31418	7H	70.61	3.32E-04	4.89	0.43	0.85	IAV.16
	12_30835	7H	70.61	3.82E-04	4.80	0.43	0.84	IAV.16
	11_10115	7H	70.64	6.17E-04	4.46	0.44	0.80	IAV.16
	SCRI_RS_154111	7H	70.68	3.82E-04	4.80	0.43	0.84	IAV.16
	SCRI_RS_112718	7H	70.75	3.32E-04	4.89	0.43	0.85	IAV.16
	SCRI_RS_164280	7H	71.46	1.61E-04	5.42	0.43	0.89	IAV.16

Annex 1: Passport information of AM-2014 panel (336 barley genotypes)

AM-2014 ID	Adaptation	Germplasm	Row type	Pedigree
AM-1	Low Input	Breeding lines	6	Alanda/5/Aths/4/Pro/TolI//Cer*2/TolI/3/5106/6/Baca'S'/3/AC253//CI08887/CI05761
AM-2	Low Input	Breeding lines	6	Zarjau/80-5151//OK84817
AM-3	Low Input	Breeding lines	6	Rhn-03/Candela
AM-4	Low Input	Breeding lines	6	Avt/Attiki//M-Att-73-337-1/3/Aths/Lignee686/4/Lignee527/NK1272//JLB70-63
AM-5	Low Input	Breeding lines	6	Arizona5908/Aths//Avt/Attiki/3/S.T.Barley/4/Aths/Lignee686/5/Maknusa
AM-6	Low Input	Breeding lines	6	Rhn-03/Alanda
AM-7	Low Input	Breeding lines	2	Clipper//WI2291*2/WI2269/7/Hml-02/5/Cq/Cm//Apm/3/12410/4/Giza134-2L/6/Clipper/Volla/3/Arr/Esp//Alger/Ceres362-1-1/4/Hml
AM-8	Low Input	Breeding lines	6	Nadawa/Rhn-03//Saida
AM-9	Low Input	Breeding lines	6	Nadawa/Rhn-03//Birka
AM-10	Low Input	Breeding lines	6	Aths/Lignee686/4/Avt/Attiki//Aths/3/Giza121/Pue
AM-11	Low Input	Variety	2	Tichedrette
AM-12	Low Input	Breeding lines	2	Soufara-02/3/RM1508/Por//WI2269/4/Hml-02/ArabiAbiad//ER/Apm/5/((GalleonxRichard)/5)xTilga
AM-13	Low Input	Breeding lines	6	Arar/Lignee527/4/Gloria'S'/Saida//Mtn'S'/EH165/3/LBIran/Una80//Lignee640
AM-14	Low Input	Breeding lines	2	GK58/3/Kc/MullersHeydla//Sls/4/Wieselbuger//Ahor1303-61//Ste/Antares
AM-15	Low Input	Breeding lines	6	Baladieldawaia/Alanda-01
AM-16	Low Input	Breeding lines	6	Rhn-03/Alanda
AM-17	Low Input	Breeding lines	2	Sadik-05//Sls/Bda
AM-18	Low Input	Breeding lines	6	Massine/Arig8
AM-19	Low Input	Breeding lines	6	As46//DeirAlla106/Strain205/3/Hyb85-6//As46/Aths*2
AM-20	Low Input	Breeding lines	6	Rhn-03/Solen
AM-21	Low Input	Breeding lines	6	Rhn-03/4/Atem/Roho//Katade/3/Aramir/ArabiAbiad
AM-22	Low Input	Breeding lines	6	Saida/6/Cita'S'/4/Apm/Rl//Manker/3/Maswi/Bon/5/Copal'S'/7/Malouh/8/Express
AM-23	Low Input	Breeding lines	6	Alanda//Ssn/Lignee640/3/QB813-2

AM-24	Low Input	Breeding lines	6	Aths/Lignee686//Arimar/Aths/3/Aths/Lignee686/4/80-5145/Rhn-05/3/As46//Giza121/Pue
AM-25	Low Input	Breeding lines	6	Rihane-03/3/As46/Aths*2//Aths/Lignee686/6/M64-76/Ben//Jo/York/3/M5/Galt//As46/4/Hj34-80/Astrix/5/M6/Robur-35-6-3
AM-26	Low Input	Breeding lines	6	Rhn-03/AC_Bacon
AM-27	High Input	Breeding lines	2	LIMON/BICHY2000//DEFRA/DESCONOCIDA-BAR
AM-28	High Input	Breeding lines	6	CANELA/5/PENCO/CHEVRON-BAR/3/ATACO/BERMEJO//HIGO/4/PETUNIA1
AM-29	High Input	Breeding lines	6	LAMOLINA96/FALCON-BAR
AM-30	High Input	Breeding lines	2	BICHY2000/SHENMAINO.3
AM-31	High Input	Breeding lines	6	BBSC/CONGONA
AM-32	High Input	Breeding lines	2	H0004700509/3H0019
AM-33	High Input	Breeding lines	6	CABUYA/MJA//PETUNIA1/5/PENCO/CHEVRON-BAR/3/ATACO/BERMEJO//HIGO/4/PETUNIA1
AM-34	High Input	Breeding lines	6	N0104700309/2H0041
AM-35	High Input	Breeding lines	6	CABUYA/MJA//PETUNIA1/5/PENCO/CHEVRON-BAR/3/ATACO/BERMEJO//HIGO/4/PETUNIA1
AM-36	High Input	Breeding lines	6	PENCO/CHEVRON-BAR/3/LEGACY//PENCO/CHEVRON-BAR
AM-37	High Input	Breeding lines	6	PENCO/CHEVRON-BAR/3/LEGACY//PENCO/CHEVRON-BAR
AM-38	High Input	Breeding lines	6	BREA/DL70//CABUYA/4/PENCO/CHEVRON-BAR//CANTUA/3/TOCTE
AM-39	High Input	Variety	6	CDC RATTAN
AM-40	High Input	Breeding lines	2	BICHY2000/SHENMAINO.3
AM-41	High Input	Variety	6	TYTO
AM-42	Low Input	Breeding lines	6	Alanda-0112/Petunia1
AM-43	Low Input	Breeding lines	2	WI3167/6/ANCA/2469//TOJI/3/SHYRI/4/ATACO/5/ALELI/7/Viringa'S'//WI2291/WI2269/3/Viringa'S'
AM-44	Low Input	Breeding lines	2	WI3167/6/ANCA/2469//TOJI/3/SHYRI/4/ATACO/5/ALELI/7/Clipper/4/Alger/Ceres//Sls/3/ER/Apm
AM-45	Low Input	Breeding lines	6	Libya/ICNBF8-614/4/Giza126/3/Lignee527/NK1272//Alanda
AM-46	Low Input	Breeding lines	2	WI3167/6/ANCA/2469//TOJI/3/SHYRI/4/ATACO/5/ALELI/7/Arar/Lignee527//Zy/DL69
AM-47	Low Input	Breeding lines	2	WI3167/6/ANCA/2469//TOJI/3/SHYRI/4/ATACO/5/ALELI/7/Schooner/Babunj//Noor68/Kataf
AM-48	Low Input	Breeding lines	2	WI3167/6/ANCA/2469//TOJI/3/SHYRI/4/ATACO/5/ALELI/7/Clipper/4/Alger/Ceres//Sls/3/ER/Apm

AM-49	Low Input	Breeding lines	6	IPA7/4/AwBlack/Aths//Arar/3/9Cr279-07/Roho/5/Alanda-01//Gerbel/Hma/3/Saida
AM-50	Low Input	Breeding lines	2	Viringa'S//WI2291/WI2269/3/H.spont.38-3/Akrash-01
AM-51	Low Input	Breeding lines	2	WI3167/6/ANCA/2469//TOJI/3/SHYRI/4/ATACO/5/ALELI/7/Schooner/Babunj//Noor68/Kataf
AM-52	Low Input	Breeding lines	2	WI3167/6/ANCA/2469//TOJI/3/SHYRI/4/ATACO/5/ALELI/7/BKFMaguelone1604/3/Apro//Sv.02109/Mari/4/Giza119/5/Shyri
AM-53	Low Input	Breeding lines	6	Aths/Lignee686//Orge905/Cr289-53-2/3/PETUNIA2/6/M64-76/Ben//Jo/York/3/M5/Galt//As46/4/Hj34-80/Astrix/5/M6/Robur-35-6-3
AM-54	High input	Breeding lines	2	Coss/OWB71080-44-1H//Viringa'S'/3/WI3180
AM-55	Low Input/ Naked barley	Breeding lines	2	Atahualpa/IraqiBlack/6/Viringa'S'//WI2291/WI2269/5/Atahualpa/4/300Union/Sv73608//Perugia/3/W28G15-1-N/Weihenstephan173
AM-56	Low Input	Breeding lines	2	WI3167/6/ANCA/2469//TOJI/3/SHYRI/4/ATACO/5/ALELI/7/Viringa'S'//WI2291/WI2269/3/Viringa'S'
AM-57	High Input	Breeding lines	2	Demhay/4/Viringa'S'//WI2291/WI2269/3/Viringa'S'
AM-58	Low Input	Breeding lines	2	WI3167/6/ANCA/2469//TOJI/3/SHYRI/4/ATACO/5/ALELI/7/Schooner/Babunj//Noor68/Kataf
AM-59	High Input	Breeding lines	2	Viringa'S/Tarida
AM-60	Low Input	Breeding lines	6	Alanda/5/Aths/4/Pro/TolI//Cer12/TolI/3/5106/6/Aths/7/Giza129
AM-61	Low Input/ Naked barley	Breeding lines	2	ICNB93-369//Atahualpa/IraqiBlack
AM-62	Low Input	Breeding lines	6	Alanda/Zafraa//Gloria'S'/Copal'S'/3/F6NB_7
AM-63	High Input	Breeding lines	2	Coss/OWB71080-44-1H//Viringa'S'/3/Viringa'S'
AM-64	Low Input	Breeding lines	2	Viringa'S//WI2291/WI2269/3/H.spont.38-3/Akrash-01
AM-65	Low Input	Breeding lines	6	Aths/Lignee686//Orge905/Cr289-53-2/3/F6NB_7
AM-66	High Input/ Low Input	Breeding lines	6	U.Sask.1766/Api//Cel/3/Weeah/4/Giza121/Pue
AM-67	High Input	Variety	6	RD2668
AM-68	Low Input	Variety	6	Furat-1
AM-69	High Input	Collection	6	Pol2
AM-70	High Input	Collection	2	Pol3
AM-71	Low Input	Breeding lines	2	Akrash//WI2291/WI2269/3/Sls/Akrash-02
AM-72	Low Input	Breeding lines	2	Tadmor//ER/Apm/3/Zanbaka
AM-73	Low Input	Breeding lines	6	Uzno-Kazakastan/4/Sonata/3/4679/105//YEA132TH

AM-74	Low Input	Breeding lines	2	AwBlack/Aths//Rhn-08/3/Malouh
AM-75	Low Input	Breeding lines	6	Baishishek/5/Nd10277/Shyri//Nd11231/Shyri/3/Azaf/4/Canela/Gob96Dh
AM-76	Low Input	Breeding lines	2	Tadmor//ER/Apm/3/Zanbaka
AM-77	Low Input	Breeding lines	2	Fedora/Express//Rhn-03
AM-78	Low Input	Breeding lines	6	Ssn/Bda//Arar/3/F2CC33MS/CI07555/4/Avt/Attiki//M-Att-73-337-1/3/Aths/Lignee686
AM-79	Low Input	Breeding lines	2	WI2198/Hml-02//INRA55-86-2/Rt1703/3/Hml
AM-80	Low Input	Breeding lines	2	GK58/3/Kc/MullersHeydla//Sls/4/Wieselbuger//Ahor1303-61//Ste/Antares
AM-81	Low Input	Breeding lines	2	Soufara-02/3/RM1508/Por//WI2269/4/Hml-02/ArabiAbiad//ER/Apm
AM-82	Low Input	Breeding lines	2	Akrash//WI2291/WI2269/3/Sls/Akrash-02
AM-83	Low Input	Breeding lines	6	Malouh//Aths/Lignee686
AM-84	Low Input	Breeding lines	2	Moroc9-75/Hml-02/5/Clipper/Volla/3/Arr/Esp//Alger/Ceres362-1-1/4/Hml
AM-85	Low Input	Breeding lines	2	Clipper//WI2291*2/WI2269/3/Furat 2
AM-86	Low Input	Breeding lines	2	3896/1-3/4/1246/1-3/3/3887/28//3892/1-3/5/Grivita/6/Antares/Ky63-1294//Marageh
AM-87	Low Input	Breeding lines	6	Nadawa/Rhn-03//Mtn-01
AM-88	Low Input	Breeding lines	6	Nadawa/Rhn-03/3/Lignee527/Rihane//Arar
AM-89	Low Input	Breeding lines	2	Soufara-02/3/RM1508/Por//WI2269/4/Hml-02 /ArabiAbiad//ER /Apm/5/ ((Galleon x Richard)/5)xTilga
AM-90	Low Input	Breeding lines	2	Tipper/ICB-102854//Alpha/Durra
AM-91	Low Input	Variety	6	Rihane 03
AM-92	Low Input	Variety	6	Local / National Check
AM-93	Low Input	Breeding lines	6	Nadawa/Rhn-03/3/Lignee527/Rihane//Arar
AM-94	Low Input	Breeding lines	2	Roho/4/Zanbaka/3/ER/Apm//Lignee131
AM-95	Low Input	Breeding lines	6	Arbayan-01//As46/Aths/3/Barjouj
AM-96	Low Input	Breeding lines	6	F6-1-Kf/6/Cita'S'/4/Apm/Rl//Manker/3/Maswi/Bon/5/Copal'S'/7/Aths/Lignee686//Orge905/Cr289-53-2
AM-97	Low Input	Breeding lines	6	F6-1-Kf/6/Cita'S'/4/Apm/Rl//Manker/3/Maswi/Bon/5/Copal'S'/7/Aths/Lignee686//Orge905/Cr289-53-2
AM-98	Low Input	Breeding lines	2	WI2269/Espe/3/WI2291/Bgs//Hml-02
AM-99	Low Input	Breeding lines	2	WI2269/3/Roho//Alger/Ceres362-1-1
AM-100	Low Input	Breeding lines	6	Sadik-02/3/Alpha/Durra//Antares/ArabiAbiad

AM-101	Low Input	Breeding lines	2	H.spont.41-1/WI3257
AM-102	Low Input	Breeding lines	2	Carina/Moroc9-75//WI3257
AM-103	Low Input	Breeding lines	2	Arar/H.spont.19-15//Hml/3/H.spont.41-1/Tadmor/4/Barque
AM-104	Low Input	Breeding lines	2	Hml-02/ArabiAbiad/3/Api/CM67//Nacta/4/WI2269/Espe/5/Mzq/Gva//PI002917/3/WI2291/WI2269
AM-105	High Input	Breeding lines	6	U.Sask.1766/Api//Cel/3/Weeah/4/Giza121/Pue
AM-106	Low Input	Breeding lines	2	Harmal-02/ArabiAbiad*2/4/Soufara-02/3/RM1508/Por//WI2269
AM-107	Low Input	Breeding lines	2	SLB44-56/Lignee131
AM-108	High Input	Breeding lines	6	IQBA07-02
AM-109	Low Input	Breeding lines	2	SLB44-56/Lignee131
AM-110	High input/ low input	Variety	6	VAMIKHOCA
AM-111	High Input	Breeding lines	6	IQBA07-22
AM-112	Low Input	Breeding lines	2	Moroc9-75//WI2291/CI01387/3/H.spont.41-1
AM-113	Low Input	Breeding lines	6	Arar/Rhn-03//Kabaa-03/4/Manal/3/Lignee527/NK1272//JLB70-63
AM-114	Low Input	Breeding lines	2	Moroc9-75//WI2291/CI01387/3/H.spont.41-1
AM-115	Low Input	Breeding lines	2	Moroc9-75//WI2291/WI2269
AM-116	Low Input	Breeding lines	2	Moroc9-75//WI2291/WI2269
AM-117	Low Input	Breeding lines	2	Clipper//WI2291*2/WI2269/5/Soufara-02/3/RM1508/Por//WI2269/4/Hml-02/ArabiAbiad//ER/Apm
AM-118	Low Input	Breeding lines	2	Clipper//WI2291*2/WI2269/5/Soufara-02/3/RM1508/Por//WI2269/4/Hml-02/ArabiAbiad//ER/Apm
AM-119	Low Input	Breeding lines	2	Clipper//WI2291*2/WI2269/5/Soufara-02/3/RM1508/Por//WI2269/4/Hml-02/ArabiAbiad//ER/Apm
AM-120	Low Input	Breeding lines	6	ArabiAbiad/Arar//H.spont.41-5/Tadmor/3/ArabiAbiad/Arar//H.spont.41-5/Tadmor
AM-121	Low Input	Breeding lines	2	Arar/H.spont.19-15//Hml/3/H.spont.41-1/Tadmor/4/Tadmor//ER/Apm
AM-122	Low Input	Breeding lines	2	Hml/Furat-2
AM-123	Low Input	Breeding lines	2	Hml/Furat-2
AM-124	Low Input	Breeding lines	2	Hml/Furat-2
AM-125	Low Input	Breeding lines	2	Hml/Furat-2

AM-126	Low Input	Breeding lines	2	WI2291/Furat-2
AM-127	Low Input	Breeding lines	2	NT111//Sonata/Arta
AM-128	Low Input	Breeding lines	2	Arta/3/Hml-02//Esp/1808-4L/5/Roho/4/Zanbaka/3/ER/Apm//Lignee131
AM-129	Low Input	Breeding lines	2	Weeah11//WI2291/Bgs/3/ER/Apm//AC253
AM-130	Low Input	Breeding lines	2	NT111//Sonata/Arta
AM-131	Low Input	Breeding lines	2	Weeah11//WI2291/Bgs/3/ER/Apm//AC253
AM-132	Low Input	Breeding lines	2	Weeah11//WI2291/Bgs/3/ER/Apm//AC253
AM-133	Low Input	Breeding lines	2	Weeah11//WI2291/Bgs/3/ER/Apm//AC253
AM-134	Low Input	Breeding lines	2	Arta/3/Hml-02//Esp/1808-4L/5/Roho/4/Zanbaka/3/ER/Apm//Lignee131
AM-135	Low Input	Breeding lines	2	WI2269/Espe/3/WI2291/Bgs//Hml-02
AM-136	Low Input	Breeding lines	2	WI2291/Roho//WI2269/3/WI2291/Bgs//Hml-02
AM-137	Low Input	Breeding lines	2	WI2291/WI2269//WI2291/Bgs/3/Hml/WI2291
AM-138	Low Input	Breeding lines	2	WI2291/Roho//WI2269/3/WI2291/Bgs//Hml-02
AM-139	Low Input	Breeding lines	2	WI2291/Roho//WI2269/3/WI2291/Bgs//Hml-02
AM-140	Low Input	Breeding lines	2	Tadmor/WI2291//Arta
AM-141	Low Input	Breeding lines	2	ChiCm/An57//Albert/3/Alger/Ceres362-1-1/4/Arta
AM-142	Low Input	Breeding lines	2	Mo.B1337/WI2291//Moroc9-75
AM-143	Low Input	Breeding lines	2	ChiCm/An57//Albert/3/Alger/Ceres362-1-1/4/Arta
AM-144	Low Input	Breeding lines	2	ChiCm/An57//Albert/3/Alger/Ceres362-1-1/4/Arta
AM-145	Low Input	Collection	6	Egypt
AM-146	Low Input	Collection	6	Tunisia
AM-147	Low Input	Collection	6	35881
AM-148	Low Input	Collection	6	Barjouj
AM-149	Low Input	Breeding lines	2	Clipper//WI2291*2/WI2269
AM-150	Low Input	Breeding lines	2	Mo.B1337/WI2291//Moroc9-75/4/Arta/3/Hml-02//Esp/1808-4L
AM-151	Low Input	Breeding lines	2	WI2291/Tadmor/3/ArabiAbiad/Arar//H.spont.41-5/Tadmor
AM-152	Low Input	Breeding lines	6	Alanda//Lignee527/Arar/3/BF891M-617/4/Alanda//Lignee527/Arar/3/BF891M-617
AM-153	Low Input	Breeding lines	2	WI3257/4/ALISO/CI3909-2//HB602/3/MOLA/SHYRI//ARUPO*2/JET
AM-154	Low Input	Breeding lines	2	Arta//Sonata/Arta

AM-155	Low Input	Breeding lines	6	Lignee527/Aths//Lignee527/NK1272
AM-156	Low Input	Breeding lines	6	Aths/Lignee686/4/Avt/Attiki//Aths/3/Giza121/Pue
AM-157	Low Input	Breeding lines	6	Rihane-03/3/As46/Aths*2//Aths/Lignee686
AM-158	Low Input	Breeding lines	6	JLB70-01/5/DeirAlla106//DL70/Pyo/3/RM1508/4/Arizona5908/Aths//Avt/Attiki/3/Ager/6/Alanda
AM-159	Low Input	Breeding lines	6	Aths/Lignee686/4/Avt/Attiki//Aths/3/Giza121/Pue
AM-160	Low Input	Breeding lines	6	Avt/Attiki//M-Att-73-337-1/3/Aths/Lignee686/4/Kabaa
AM-161	Low Input	Breeding lines	6	Courlis/Rihane-03//Rhn-03
AM-162	Low Input	Breeding lines	6	Avt/Attiki//M-Att-73-337-1/3/Aths/Lignee686/4/Kabaa
AM-163	Low Input	Breeding lines	6	Merzaga(Orge077)/Alanda-01
AM-164	Low Input	Breeding lines	6	Alanda/Hamra//Alanda-01
AM-165	Low Input	Breeding lines	6	Nadawa/Rhn-03/3/Lignee527/Rihane//Arar
AM-166	Low Input	Breeding lines	6	Rhn-03//Lignee527/As45/4/Manitou/3/Arbayan-01//CI07117-9/DeirAlla106
AM-167	Low Input	Breeding lines	6	Rhn-03//Lignee527/As45/3/Y25-3-1
AM-168	Low Input	Breeding lines	6	Nadawa/Rhn-03/3/Lignee527/Rihane//Arar
AM-169	Low Input	Breeding lines	6	Rhn-03/3/Mr25-84/Att//Mari/Aths*3-02/4/Alanda-01//Gerbel/Hma/3/Saida
AM-170	Low Input	Breeding lines	6	IPA7/4/AwBlack/Aths//Arar/3/9Cr279-07/Roho/5/Rhn-03//Lignee527/As45
AM-171	Low Input/ High Input	Breeding lines	6	JLB70-01/Asher//Russ94-1
AM-172	Low Input	Breeding lines	2	Clipper//WI2291*2/WI2269/5/Soufara-02/3/RM1508/Por//WI2269/4/Hml-02/ArabiAbiad//ER/Apm
AM-173	Low Input	Breeding lines	6	Mari/Aths*2//Avt/Attiki/3/Aths/Lignee686/4/Arar//Hr/Nopal
AM-174	Low Input	Breeding lines	6	Tadmor//ER/Apm/3/H.spont.38-3/Akrash-01
AM-175	Low Input	Breeding lines	6	Lignee527/Chn-01//Alanda/5/Arizona5908/Aths//Avt/Attiki/3/S.T.Barley/4/Aths/Lignee686
AM-176	Low Input	Breeding lines	6	Aths/Lignee686/4/Avt/Attiki//Aths/3/Giza121/Pue
AM-177	Low Input	Breeding lines	6	Rihane-03/3/As46/Aths*2//Aths/Lignee686/4/Alanda-01
AM-178	Low Input	Breeding lines	6	Rihane-03/3/As46/Aths*2//Aths/Lignee686/4/Alanda-01
AM-179	Low Input	Breeding lines	6	Lignee527/Chn-01//Alanda/3/As57/Kc
AM-180	Low Input	Breeding lines	6	Avt/Attiki//M-Att-73-337-1/3/Aths/Lignee686/5/AwBlack/Aths//Arar/3/9Cr279-07/Roho/4/Aths
AM-181	Low Input	Breeding lines	6	Lignee527/Aths//Aths/Lignee686/3/Aths

AM-182	Low Input	Breeding lines	6	Lignee527/Aths//Aths/Lignee686/3/Aths
AM-183	Low Input	Breeding lines	6	3201-3/Perugia//Berolina/Weihenstephan173/4/Aths/Lignee686/3/DeirAlla106/Lignee527//Assala
AM-184	Low Input	Breeding lines	6	Lignee527/Aths//Aths/Lignee686/3/Alanda
AM-185	Low Input	Breeding lines	6	Lignee527/Aths//Aths/Lignee686/3/Alanda
AM-186	Low Input	Breeding lines	2	WI2291/4/7028/2759/3/69-82//Ds/Apro
AM-187	Low Input	Breeding lines	6	Lignee527/NK1272//UL76252/Jaidor/3/Alanda
AM-188	Low Input	Breeding lines	6	AwBlack/Aths//Arar/3/9Cr279-07/Roho/4/CompCr229//As46/Pro/3/DeirAlla106//DL71/Strain205
AM-189	Low Input	Breeding lines	6	Avt/Attiki//M-Att-73-337-1/3/Aths/Lignee686/4/CYDBA89#49/3/Ssn/Bda//Arar
AM-190	Low Input	Breeding lines	6	Ghinneri(smooth_awns)/5/AwBlack/Aths//Arar/3/9Cr279-07/Roho/4/Aths
AM-191	Low Input	Breeding lines	6	Ghinneri(smooth_awns)/Alanda
AM-192	Low Input	Breeding lines	6	Ghinneri(smooth_awns)/Alanda
AM-193	Low Input	Breeding lines	6	AwBlack/Aths//Arar/3/9Cr279-07/Roho/6/Alanda-01/5/CI01021/4/CM67/U.Sask.1800//Pro/CM67/3/DL70
AM-194	Low Input	Breeding lines	6	Alanda/Hamra/4/Avt/Attiki//M-Att-73-337-1/3/Aths/Lignee686
AM-195	Low Input	Breeding lines	6	Marar/4/CompCr229//As46/Pro/3/Srs
AM-196	Low Input	Breeding lines	6	AwBlack/Aths//Arar/3/9Cr279-07/Roho/6/Alanda-01/5/CI01021/4/CM67/U.Sask.1800//Pro/CM67/3/DL70
AM-197	Low Input	Variety	6	Rihane 03
AM-198	Low Input	Breeding lines	6	AwBlack/Aths//Arar/3/9Cr279-07/Roho/6/Alanda-01/5/CI01021/4/CM67/U.Sask.1800//Pro/CM67/3/DL70/7/Aths
AM-199	Low Input	Breeding lines	6	BF891M-617/4/Hma-02//11012-2/CM67/3/Arar/5/BlackTaridaN
AM-200	Low Input	Breeding lines	6	Alanda-0112/Petunia1
AM-201	Low Input	Breeding lines	6	Alanda/5/Aths/4/Pro/TolI//Cer12/TolI/3/5106/6/Aths/7/Giza129
AM-202	High Input	Breeding lines	6	PETUNIA 2/3/AGAVE/SUMBARD400//MARCO/4/PETUNIA 1/5/TRA-B/1038//PETUNIA 1/3/PENCO/6/BLLU
AM-203	Low Input	Breeding lines	2	WI3167/6/ANCA/2469//TOJI/3/SHYRI/4/ATACO/5/ALELI/7/Schooner/Babunj//Noor68/Kataf
AM-204	Low Input	Breeding lines	2	ICNB93-369/IRAN(Kordistan)
AM-205	Low Input	Breeding lines	6	Libya/ICNBF8-614/4/Ssn/Badia//Arar/3/Gloria'S'/Copal'S'
AM-206	Low Input/ Naked barley	Breeding lines	2	ICNB-369/WI3295

AM-207	Low Input/ Naked barley	Breeding lines	2	Viringa'S//WI2291/WI2269/3/H.spont.38-3/Akrash-01
AM-208	Low Input/ Naked barley	Breeding lines	6	DD-21/4/ALISO/CI3909.2//HB602/3/MOLA/SHYRI//ARUPO*2/JET
AM-209	Low Input/ Naked barley	Breeding lines	6	WI3180/4/ALISO/CI3909.2//HB602/3/MOLA/SHYRI//ARUPO*2/JET
AM-210	Low Input/ Naked barley	Breeding lines	6	Atahualpa/3/Arar/Lignee527//Zy/DL69
AM-211	Low Input	Breeding lines	6	WI3167/6/ANCA/2469//TOJI/3/SHYRI/4/ATACO/5/ALELI/7/Schooner/Babunj//Noor68/Kataf
AM-212	Low Input/ Naked barley	Breeding lines	2	Rt013/Nainaa/4/Lignee527/Chn-01//Gustoe/3/Atahualpa
AM-213	High Input/ Naked barley	Breeding lines	6	PETUNIA 1/5/POST/COPAL//GLORIA-BAR/COME/3/SIND89A-148/4/CARDO/6/GLORIA-BAR/COPAL//BLLU/3/PETUNIA 1/7/PINON
AM-214	Low Input/ Naked barley	Breeding lines	6	Aths/Lignee686/5/Alanda-01/4/WI2291/3/Api/CM67//L2966-69/6/Atahualpa/7/CANELA/GOB//ALELI
AM-215	Low Input/ Naked barley	Breeding lines	6	WI3159/5/MOLA/SHYRI//ARUPO*2/JET/3/ALELI/4/MOLA/SHYRI//ARUPO*2/JET/3/CONDOR-BAR
AM-216	High Input/ Naked barley	Breeding lines	2	LIMON/BICHY2000/4/AZAF/3/ARUPO/K8755//MORA
AM-217	High Input/ Naked barley	Breeding lines	6	Arupo'S'12/3/PI002325/Maf102//Cossack/4/Viringa'S'/5/Atahualpa
AM-218	Low Input/ Naked barley	Breeding lines	6	ICNB93-369/IRAN(Kordistan)
AM-219	Low Input/ Naked barley	Breeding lines	6	Atahualpa//Alanda-01/Hamra/3/Keel
AM-220	High Input/ Naked barley	Breeding lines	6	BICHY 2000(6H)/ZIGZIG
AM-221	High Input/ Naked barley	Breeding lines	6	TOCTE/PETUNIA 2//PETUNIA 1
AM-222	Low Input/ Naked barley	Breeding lines	6	WI3167/6/ANCA/2469//TOJI/3/SHYRI/4/ATACO/5/ALELI/7/Schooner/Babunj//Noor68/Kataf
AM-223	Low Input/ Naked barley	Breeding lines	6	Avt/Attiki//M-Att-73-337-1/3/Aths/Lignee686/4/F6NB2_Khomes

AM-224	High Input/ Naked barley	Breeding lines	6	DD-21/4/ALISO/CI3909.2//HB602/3/MOLA/SHYRI//ARUPO*2/JET
AM-225	Low Input/ Naked barley	Breeding lines	2	DD-21/3/Harmal-02/ArabiAbiad//ER/Apm
AM-226	Low Input/ Naked barley	Breeding lines	6	WI3167/6/ANCA/2469//TOJI/3/SHYRI/4/ATACO/5/ALELI/7/Schooner/Babunj//Noor68/Kataf
AM-227	High Input/ Naked barley	Breeding lines	6	Arupo'S'12/3/PI002325/Maf102//Cossack/4/Viringa'S'/5/WI3180
AM-228	High Input/ Naked barley	Breeding lines	2	DD-21/Orzo
AM-229	High Input/ Naked barley	Breeding lines	2	Alanda/Zafraa//Gloria'S'/Copal'S'/3/F6NB_7
AM-230	Low Input	Variety	6	Alanda 01
AM-231	Low Input	Variety	2	WI2291
AM-232	Low Input	Variety	2	Harmal
AM-233	Low Input	Variety	2	Nawair-01
AM-234	Low Input	Variety	6	Momtaz
AM-235	Low Input	Variety	6	Manal
AM-236	High Input	Breeding lines	6	P.STO/3/LBIRAN/UNA80//LIGNEE640/4/BLLU/5/PETUNIA 1
AM-237	High Input	Breeding lines	6	H96109010
AM-238	High Input	Breeding lines	6	H96117004
AM-239	High Input	Variety	6	M122 (Quest)
AM-240	High Input	Breeding lines	6	PENCO/CHEVRON-BAR
AM-241	High Input /low input	Variety	6	PETUNIA 1
AM-242	High Input	Variety	6	CIRUELO
AM-243	High Input	Breeding lines	6	ESMERALDA/LEGACY/6/P.STO/3/LBIRAN/UNA80//LIGNEE640/4/BLLU/5/PETUNIA 1
AM-244	High Input	Breeding lines	6	QUINA/MJA//SCARLETT
AM-245	High Input	Breeding lines	6	PENCO/CHEVRON-BAR//ATAH92/GOB
AM-246	High Input	Breeding lines	6	ESMERALDA/3/SLLO/ROBUST//QUINA/4/M104

AM-247	High Input	Breeding lines	6	ATACO/BERMEJO//HIGO/3/CALI92/ROBUST/4/PETUNIA1/5/PETUNIA/CHINIA/3/ATACO /BERMEJO//HIGO/6/ZIGZIG/3/M9846//CCXX14.ARZ3/PACO
AM-248	High Input	Breeding lines	6	P.STO/3/LBIRAN/UNA80//LIGNEE640/4/BLLU/5/PETUNIA 1/6/M9846//CCXX14.ARZ3/PACO/3/PALTON
AM-249	High Input	Breeding lines	6	P.STO/3/LBIRAN/UNA80//LIGNEE640/4/BLLU/5/PETUNIA 1/6/ZIGZIG/4/EGYPT4/TERAN78//P.STO/3/QUINA
AM-250	High Input	Breeding lines	6	P.STO/3/LBIRAN/UNA80//LIGNEE640/4/BLLU/5/PETUNIA 1/6/M111/7/LEGACY/3/SVANHALSBAR/MSEL//AZAF/GOB24DH
AM-251	High Input	Breeding lines	6	SARA1-BAR/CAPUCHONA 20
AM-252	High Input	Breeding lines	6	PETUNIA 2/M111
AM-253	Low Input/ Landrace	Variety	6	C-84
AM-254	Low Input/ Landrace	Variety	6	C 50
AM-255	Low Input/ Landrace	Variety	6	K 12
AM-256	Low Input/ Landrace	Variety	6	K 14
AM-257	High Input	Variety	6	JYOTI
AM-258	Low Input /Feed	Variety	6	LAKHAN (K226)
AM-259	High Input/ Feed	Variety	6	PL 172
AM-260	Low Input/ Food	Variety	6	GEETANJALI (K1149)
AM-261	High Input /Feed	Variety	6	PL426
AM-262	Low Input /Feed	Variety	6	RD2508
AM-263	High Input/ Feed	Variety	6	RD2552
AM-264	High Input/ Feed	Variety	6	RD2624

AM-265	High Input /Feed	Variety	6	RD2592
AM-266	High Input/ Salinity tolerant	Variety	6	NDB1173
AM-267	Low Input /Feed	Variety	6	RD2660
AM-268	High Input/ Malting	Variety	2	DWR28
AM-269	High Input/ Malting	Variety	2	RD2668
AM-270	High Input/ Malting	Variety	2	DWRUB52
AM-271	Low Input/ Naked barley	Breeding lines	2	ICNB93-369/IRAN(Kordistan)
AM-272	Low Input/ Naked barley	Breeding lines	2	WI3167/6/ANCA/2469//TOJI/3/SHYRI/4/ATACO/5/ALELI/7/LEO-B/CANELA//GOB96DH
AM-273	Low Input/ Naked barley	Breeding lines	2	WI3167/6/ANCA/2469//TOJI/3/SHYRI/4/ATACO/5/ALELI/7/LEO-B/CANELA//GOB96DH
AM-274	Low Input/ Naked barley	Breeding lines	2	WI3167/4/ALISO/CI3909.2//HB602/3/MOLA/SHYRI//ARUPO*2/JET
AM-275	Low Input/ Naked barley	Breeding lines	2	WI3167/6/ANCA/2469//TOJI/3/SHYRI/4/ATACO/5/ALELI/7/LEO-B/CANELA//GOB96DH
AM-276	Low Input/ Naked barley	Breeding lines	2	WI3167/6/ANCA/2469//TOJI/3/SHYRI/4/ATACO/5/ALELI/7/Schooner/Babunj//Noor68/Kataf
AM-277	Low Input/ Naked barley	Breeding lines	2	WI3257/4/ALISO/CI3909.2//HB602/3/MOLA/SHYRI//ARUPO*2/JET
AM-278	Low Input/ Naked barley	Breeding lines	2	DD-21/Mundah
AM-279	Low Input/ Naked barley	Breeding lines	2	WI3167/6/ANCA/2469//TOJI/3/SHYRI/4/ATACO/5/ALELI/7/Schooner/Babunj//Noor68/Kataf
AM-280	Low Input/ Naked barley	Breeding lines	2	WI3167/6/ANCA/2469//TOJI/3/SHYRI/4/ATACO/5/ALELI/7/Schooner/Babunj//Noor68/Kataf

AM-281	Low Input/ Naked barley	Breeding lines	2	Viringa'S/Tarida
AM-282	Low Input/ Naked barley	Breeding lines	2	Viringa'S//WI2291/WI2269/3/H.spont.38-3/Akrash-01
AM-283	Low Input/ Naked barley	Breeding lines	6	Aths/Lignee686//Orge905/Cr289-53-2/3/F6NB_7
AM-284	Landrace/ Extra Early (Belarus)	Collection	6	IG:154361
AM-285	Landrace/ Extra Early (Nepal)	Collection	6	IG:153841
AM-286	Landrace/ Extra Early (Nepal)	Collection	6	IG:153839
AM-287	Landrace/ Extra Early (Nepal)	Collection	6	IG:153842
AM-288	Landrace/ Extra Early (Armenia)	Collection	2	IG:155491
AM-289	Landrace/ Extra Early (Belarus)	Collection	6	IG:154360
AM-290	Landrace/ Extra Early (Nepal)	Collection	6	IG:153846
AM-291	Landrace/ Extra Early (Nepal)	Collection	6	IG:153849
AM-292	Landrace/ Extra Early (Belarus)	Collection	6	IG:154357

AM-293	Landrace/ Extra Early (Mangolia)	Collection	6	IG:156345
AM-294	Landrace/ Extra Early (Armeia)	Collection	6	IG:155493
AM-295	Landrace/ Extra Early (Mangolia)	Collection	6	IG:156369
AM-296	High input	Collection	2	MXB.486
AM-297	High input/ low input	Variety	2	Stirling
AM-298	Landrace	Collection	2	CI19819
AM-299	Low input	Variety	2	Esperance Orge 289
AM-300	High input/ low input	Variety	6	Arimont
AM-301	High input/ low input	Variety	6	Kombar
AM-302	High input/ low input	Collection	2	TR03189
AM-303	High Input	Variety	2	Baudin
AM-304	Landrace	Collection	2	CI3576
AM-305	Landrace	Collection	2	CI11456
AM-306	High Input	Variety	6	Steptoe
AM-307	High input	Variety	6	Beecher
AM-308	High input/ low input	Variety	6	Cape
AM-309	Low Input	Collection	2	BYDV-013
AM-310	Landrace	Collection	6	CI5286
AM-311	High input/ low input	Variety	2	Chebec

AM-312	High input/ low input	Variety	6	Jet
AM-313	High Input	Variety	2	Skiff
AM-314	High input	Variety	2	Yangsimai 3
AM-315	High Input	Variety	2	Herta
AM-316	High Input	Variety	2	Summit
AM-317	High Input	Variety	2	Galleon
AM-318	High Input	Variety	2	Keel
AM-319	High input/ low input	Variety	2	Yagan
AM-320	High input/ low input	Collection	2	TR250
AM-321	Low Input	Collection	6	ICARDA SN326
AM-322	Landrace	Collection	6	CI5791
AM-323	High input/ low input	Collection	6	KB35
AM-324	Landrace	Collection	6	CI9776
AM-325	Landrace	Variety	6	Haruna Nijo
AM-326	Landrace	Collection	6	CI7584
AM-327	High input/ low input	Variety	2	Torrens
AM-328	low input	Variety	6	Coast
AM-329	Landrace	Collection	6	CI9214
AM-330	Low Input	Breeding lines	6	Aths/Lignee686/4/Avt/Attiki//Aths/3/Giza121/Pue
AM-331	Low Input	Breeding lines	6	Rihane-03/3/Mr25-84/Att//Mari/Aths*3-02/4/Alanda-01
AM-332	Low Input	Breeding lines	6	Saida/6/Cita'S'/4/Apm/Rl//Manker/3/Maswi/Bon/5/Copal'S'/7/Malouh/8/Alanda-01
AM-333	Low Input	Breeding lines	6	Nadawa/Rhn-03/3/Lignee527/Rihane//Arar
AM-334	Low Input	Breeding lines	6	Nadawa/Rhn-03//QB813.2
AM-335	Low Input	Breeding lines	6	WI2291/Roho//WI2269/3/Arta
AM-336	Low Input	Breeding lines	2	Clipper//WI2291*2/WI2269/5/Soufara-02/3/RM1508/Por//WI2269/4/Hml-02/ArabiAbiad//ER/Apm