



Review on Molecular Marker Used in Barely Breeding

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<p>Abstract: Barley (<i>Hordeum vulgare</i>) is the largest cereal crop in the world, mostly used for animal feed, food, and industrial utilization. In its endosperm, the nutrients like starch and protein are stored and it determine the barely quality. Many barley-breeding programs around the world have used accessions from Ethiopia as parents in their crosses. Ethiopian landraces are known for several important traits, including resistance to powdery mildew. The NDSU breeding program mainly focuses on developing two-rowed and six-rowed malt barley cultivars adaptable to the northern Great Plains of the USA while the ICARDA breeding program develops cultivars for diverse agro-ecologies, including dry land and high rainfall regions.</p> <p>Keywords: Marker, QTL Mapping, Marker Assisted Selection, Malt Barely, Linkage.</p> <p>Copyright © 2024 The Author(s): This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CC BY-NC 4.0) which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.</p>	<p>REVIEW PAPER</p>
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1. INTRODUCTION

Barley (*Hordeum vulgare*) is the largest cereal crop in the world, mostly used for animal feeding, food, and industrial utilization (Bond *et al.*, 2015; FAOSTAT¹). It is about 30% of the product is globally used for malting, breeding barley varieties with higher-quality of malt for processing is useful goal (Bond *et al.*, 2015; Walker and Panozzo, 2016; Kochevenko *et al.*, 2018).

Many barley-breeding programs around the world have used accessions from Ethiopia as parents in their crosses. Ethiopian landraces are known for several important traits, including resistance to powdery mildew (caused by *Erysiphe graminis* DC. f. sp. *hordei* Em. Marchal), leaf rust (caused by *Puccinia hordei* Otth), loose smut (caused by *Ustilago tritici* (Pers.) Rostr.), barley yellow dwarf virus (BYDV), and barley stripe mosaic virus (BSMV); and high lysine content (Adugna, 2011; Spies *et al.*, 2012; Munoz *et al.*, 2014). The Ethiopian Institute of Biodiversity Conservation (IBC) has conserved > 15,000 barley accessions, with nearly 67% being landraces collected in Ethiopia (Adugna, 2011). The diversity in Ethiopian barley landraces stems from the country's diverse agro-ecologies, diverse socio-cultural situations, and wide ranges of utilization of barley for food, feed, and alcoholic beverages.

Generally, the barley-breeding program utilizes landrace collections, exotic introductions, and lines from local crossing programs to develop cultivars for diverse

production systems, including late, early, and 'Belg' (short growing season with planting in February to March). As a result, the Ethiopian barley-breeding program's germplasm has maintained high levels of genetic diversity. The barley-breeding programs of North Dakota State University (NDSU) and ICARDA have unique germplasm that reflects their breeding objectives.

Mapping of QTL for traits of breeding is an interesting precursor for MAS or molecular-marker based breeding. For showing malt quality QTL, different mapping approaches have been used, including bi-parental mapping populations (Emebiri *et al.*, 2004; Zhou *et al.*, 2012; Islamovic *et al.*, 2014); association mapping (Cai *et al.*, 2013; Matthies *et al.*, 2014; Mohammadi *et al.*, 2015); and fine mapping using chromosome substitution lines (Gao *et al.*, 2004) and wild barley introgression lines (Schmalenbach and Pillen, 2009). Linkage and association mapping are the most commonly used QTL mapping approaches (Abdurakhmonov and Abdugarimov, 2008). There is an information gap to mapping using molecular markers. There for the objective of this paper is to review Molecular Marker used in barely breeding.

2. Molecular Marker System

A genetic marker is defined as a chromosomal landmark that allows tracing a specific region of DNA (Semagn *et al.*, 2006). Genetic markers and the genes they mark are close together in the same chromosome that tends to stay together in each generation.

Several maps have been developed for barley using cytogenetic techniques (mainly using trisomy's), isozymes, morphological markers, and a range of DNA markers including SSR, DArT, and SNPs (Hussain, 2006; Wenzl *et al.*, 2006; Varshney *et al.*, 2007; Suzcs *et al.*, 2009; Munoz *et al.*, 2011). According to Gupta *et al.*, (2001), SNPs are more abundant in plant systems compared to the human genome on which they were initially applied. Close *et al.*, (2009) selected 3,072 SNPs to fill two 1,536-SNP "production" assays (BOPA1 and BOPA2). Examination of USA breeding materials with these SNP markers provided excellent coverage and sensitivity for detection of minor alleles (Close *et al.*, 2009). Most importantly, SNPs provide the ultimate form of molecular markers because a nucleotide base is the smallest unit of inheritance (Edwards *et al.*, 2007). Edwards *et al.*, (2007) estimated the SNP frequency in barley to be one SNP every 27 to 240 bp. Even if SNPs at any particular site can in principle involve four different nucleotide variants, they are generally biallelic in nature. Edwards *et al.*, (2007) pointed out that SNP markers are abundant in the genome and low in mutation rate. However, the abundance of SNP markers in the genome compensates for their biallelic nature. These features make SNPs excellent markers in studying genome evolution, map-based positional cloning, studying complex genetic traits, genetic mapping, detection of marker-trait associations, and assessment of genetic relationships between individuals (Edwards *et al.*, 2007).

Elshire *et al.*, (2011) developed a genotyping-by-sequencing (GBS) approach that is suitable for population studies, germplasm characterization, breeding, and trait mapping in diverse organisms. GBS is the latest application of next-generation sequencing protocols for the purposes of discovering SNPs in a variety of crop species and populations (Spindel *et al.*, 2013). The GBS procedure reduces cost per sample by sequencing only subsets of genomic regions targeted by restriction enzymes (Elshire *et al.*, 2011). The value of sequencing restriction site-associated genomic DNA (i.e., RAD tags) for high density SNP discovery and genotyping was first demonstrated by Baird and coworkers in 2008 (Elshire *et al.*, 2011). Alternatively, a series of polymerase chain reactions (PCR) can also be used instead of restriction enzymes to sample specific regions of the genome to sequence.

Molecular markers serve a variety of purposes relevant to crop improvement and genetic study. The applications include QTL mapping (Jones *et al.*, 1997; Gupta *et al.*, 2001; Sreenivasulu *et al.*, 2008; Edwards *et al.*, 2007; Wang *et al.*, 2012), genetic diversity analysis (Mohammadi and Prasanna, 2003), population structure studies (Wang *et al.*, 2012), and phylogenetic and comparative genomics analyses (Whitkus *et al.*, 1992). Molecular markers are also useful tools to overcome linkage drag and background genetic effect problems associated with utilization of landraces and wild types in

crop improvement (Hussain, 2006). In crop breeding, molecular markers are useful in MAS methods, such as F2 enrichment, marker-assisted back crossing, marker-assisted recurrent selection (Bernardo and Charcosset, 2006), and genomic selection (Bernardo, 2009; Bernardo, 2010; Bernardo, 2013; Massman *et al.*, 2012).

2.1 QTL Mapping

Two commonly used QTL mapping approaches are linkage mapping and association mapping (Abdurakhmonov and Abdurkarimov, 2008; Cavanagh *et al.*, 2008; Sreenivasulu *et al.*, 2008). The linkage mapping approach utilizes a bi-parental mapping population segregating for the trait(s) of interest whereas association mapping utilizes a well-chosen natural population of lines, accessions, or cultivars referred to as the "mapping panel". Both linkage analysis and association mapping rely on co-inheritance of functional polymorphisms and neighboring DNA variants (Zhu *et al.*, 2008), ultimately identifying genotype-phenotype associations that lead to discovery of QTL that are responsible for phenotypic variation (Abdurakhmonov and Abdurkarimov, 2008; Zhu *et al.*, 2008; Myles *et al.*, 2009). The three basic requirements to map QTL are a genetic map of variable markers, a population with which to follow the segregation of these markers, and trait measurements on individuals of the population (Slate, 2005).

2.1.1 Linkage Disequilibrium (LD)

Linkage equilibrium (LE) and linkage disequilibrium (LD) are important terms to describe linkage relationships in population genetics (Abdurakhmonov and Abdurkarimov, 2008). Linkage equilibrium is the random association of alleles at different loci in a population. On the contrary, LD is the non-random association of alleles at different loci in a population. Linkage disequilibrium does not necessarily imply genetic linkage, and it can occur between physically unlinked loci (Flint-Garcia, 2003; Mackay and Powell, 2006; Abdurakhmonov and Abdurkarimov, 2008). Linkage refers to the correlated inheritance of loci due to their physical connection on a chromosome whereas LD refers to the correlation of alleles in a population (Flint-Garcia, 2003). In generally, LD creates the basis for the construction of genetic maps and the localization of genetic loci for a variety of characters (Hussain, 2006). Association mapping particularly relies on LD decay (Mackay and Powell, 2006).

2.1.2 Linkage (Family) Mapping

Linkage mapping is a commonly employed QTL mapping method to explain phenotypic variation in terms of simple changes in DNA sequence in experimental populations created by bi-parental crosses (Myles *et al.*, 2009). Linkage mapping involves six general steps (Abdurakhmonov and Abdurkarimov, 2008): (1) developing an experimental population (F2, doubled-haploid, backcross, near-isogenic lines, and recombinant-inbred lines), (2) phenotyping (collecting

data on traits) across environments, (3) genotyping using markers that identify polymorphisms in the parents, (4) constructing linkage maps using molecular markers, (5) statistically correlating phenotypic data with positioned markers, and (6) identifying QTL regions affecting a trait of interest. Some of the advantages of linkage mapping include identification of low frequency functional alleles and application when there is a strong relatedness problem (Myles *et al.*, 2009).

2.1.3 Association (Population) Mapping in Barely

Association mapping involves searching for genotype-phenotype correlations (i.e. marker-trait associations) in unrelated individuals taken from a natural population (Myles *et al.*, 2009). The method results in localization of QTL based on the strength of the correlation between mapped genetic markers and traits of interest (Mackay and Powell, 2006). Decay of LD is the basis for association mapping (Mackay and Powell, 2006). Association mapping can lead to the most effective utilization of ex-situ conserved natural genetic diversity (Abdurakhmonov and Abdugarimov, 2008).

The association mapping panel could be composed of three types of populations, namely; germplasm bank collections, elite breeding materials, and synthetic populations (Bresseghello and Sorrells, 2006).

Family-based linkage (FBL) mapping is a special case of association mapping in which the mapping population is established from a small number of founders (Mackay and Powell, 2006). Cavanagh *et al.*, (2008) also discussed the importance of Multi-parent Advanced Generation Inter-cross (MAGIC) populations derived from elite breeding germplasm for gene-trait analysis in crop species.

2.2 Yield and Yield-Related Traits

2.2.1 Mapped QTL for Different Traits in Barley

Grain yield improvement is the primary objective in many cereal-breeding programs (Welsh, 1981) and it can be defined in terms of the product of three yield components, i.e.; number of spikes per unit area, number of kernels per spike, and kernel weight (Nickell and Grafius, 1969). Currently, there is growing interest in the application of molecular marker information closely related to important traits in breeding programs. The first RFLP marker map for barley developed two decades ago was ultimately used to map agronomic, quality, and disease resistance traits (Sreenivasulu *et al.*, 2008). Hussain (2006) reviewed several articles on QTL mapping in barley and discussed chromosomal location of 16 agronomic traits. According to review by Sreenivasulu *et al.*, (2008), 1000-kernel weight and kernel number per spike were mapped to all chromosomes except 1H and 7H, respectively. The other yield component, spike number per unit area, was mapped to chromosome 3H. For plant height and days to

heading, they reported several QTL in all seven chromosomes.

Xue *et al.*, (2010) did linkage analysis for yield and yield components under waterlogged and well drained conditions using 156 doubled-haploid lines derived from the cross 'Yerong' (waterlogging-tolerant) x 'Franklin' (waterlogging-sensitive). Using a genetic linkage map of 496 DArT, 80 AFLP, and 28 microsatellite markers, they identified 31 QTL for kernel weight, grains per spike, spikes per plant, spike length, and grain yield, with individual QTL explaining 4.7% to 55.3% of the phenotypic variability. Interestingly, most of those QTL with larger effects were detected in the same region of chromosome 2H, indicating tight linkage or pleiotropic effects of the gene(s) controlling the traits. They also identified some unique QTL under waterlogging conditions, which implied that different markers might be used in selecting cultivars under such conditions.

2.2.2 Disease Resistance Traits in Barely

Selection for disease resistance has been equally important like improving crops for yield and yield components. Diseases can affect yield and quality of the product as well as leaving myco-toxins on the grain. Fusarium head blight (FHB; incited by *Fusarium graminearum* Schwabe), leaf scald (incited by *Rhynchosporium secalis* (Oudem.) J. J. Davis, the net form net blotch (incited by *Drechslera teres* (Sacc.) Shoemaker) and spot blotch (incited by *Cochliobolus sativus* (Ito & Kuribayashi) Drechs. ex Dastur) are among the major fungal diseases in barley (Hussain, 2006), with resistance to each by one to 14 genes.

Fusarium head blight is economically important disease that can cause yield and quality reduction in cereals, like in wheat and barley (Ma *et al.*, 2000; Dahleen *et al.*, 2003). The U.S. Government Accounting Office (GAO) estimated total losses due to FHB in the upper-Midwest USA exceeded \$200 million from 1993-1997 (U.S. GAO, 1999). Nganje *et al.*, (2001) estimated losses of \$136 million in the same region from 1998-2000.

Leaf scald is one of the most severe diseases of barley in the highlands of Ethiopia where precipitation is high and temperature is low during the cropping season. Yield losses can range from 21% to 67%, and it also affects grain quality and ultimately the price paid for the grain (Kiros *et al.*, 2004; Zhan *et al.*, 2008).

3. CONCLUSION

Barley (*Hordeum vulgare*) is the most important cereal crop in the world, widely used for animal feeding feed, food, and industrial utilization. A genetic marker is a chromosomal landmark that allows tracing a specific region of DNA. Genetic markers and the genes they mark are close together in the same chromosome that tends to stay together in each

generation. Molecular markers serve a variety of purposes relevant to crop improvement and genetic study. Molecular markers are also useful tools to overcome linkage drag and background genetic effect problems associated with utilization of landraces and wild types in crop improvement. In crop breeding, molecular markers are useful in MAS methods, such as F2 enrichment, marker-assisted back crossing, and marker-assisted recurrent selection.

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