



Barley (*Hordeum vulgare* L.) Classification, Diversity and Health Benefits: A Review

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ABSTRACT

Barley is one of the world's most significant crops, providing millions of people with food and related products. A crop with a huge genome, as well as a vast number of varieties and accessions, is extremely rare. Systematic molecular evaluation is required for both effective utilization of available diversity in breeding and appropriate genetic diversity conservation. Despite the use of fungicides and resistant types, diseases continue to pose a severe danger to barley production. Barley is an important cereal grain that is not widely consumed by children. It's an old grain with a long list of health benefits, including weight loss, lower blood pressure, lower cholesterol, lower blood glucose in Type 2 diabetes, and prevention of colon cancer. Due to the ongoing crossing with elite genotypes, genetic variability is diminishing day by day, potentially increasing vulnerability to adverse climatic changes and limiting the potential for further improvement. Morphological indicators, biochemical or isozyme markers, and DNA-based markers are all used to determine genetic diversity. For genotyping in various crops, a large range of molecular markers is available. Due to their ease of application, abundance, co-dominance, low cost, and high polymorphism index, SSR markers are potential markers of choice for diversity assessment.

Keywords: Barley; Biochemical; Climate; Health; Isozyme; Markers

INTRODUCTION

Barley was one of the first and earliest crops, and it is the only cultivated species of the *Hordeum* genus, which contains about 32 species and 45 taxa. Barley (*Hordeum vulgare* L., $2n=2x=14$) is a self-pollinating diploid cereal crop domesticated from its wild relative, *Hordeum spontaneum*, with a genome size of 5.1 Gbp and more than 80% repetitive elements (Sato 2020). It belongs to the Poaceae family. In terms of planting area, it is the fourth most important cereal crop after maize (*Zea mays* L.), rice (*Oryza sativa* L.), and wheat (*Triticum spp.*)

Barley is grown on nearly 48 million hectares worldwide, with a grain production of over 141 million tonnes, primarily in temperate regions. In 2016, global barley production totaled more than 141 million tonnes, with Ukraine, Canada, France, Ger-

many, Australia, and Russia leading the way. During the crop season 2018-19, barley was grown on 0.66 million hectares in India, with production and average productivity of 1.73 million tonnes and 26.17 q/ha, respectively. Punjab has the highest average crop productivity in barley (3800 kg/ha), followed by Haryana (3204 kg/ha), Rajasthan (2950 kg/ha), and Uttar Pradesh (2801 kg/ha).

BENEFITS OF BARLEY CONSUMPTION

Global food demand is increasing, and meeting this demand is one of agriculture's most difficult challenges during this time of climate change. It is one of the most widely adapted crops grown in over 100 countries, with the most diverse genetic basis [1]. It thrives in a variety of environmental conditions. It is widely used for human consumption, animal feed, beer brewing

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[2], bakery industries, and processing into barley vinegar, biscuits, malted food drinks, sugar confectionery, and functional compounds such as vitamins B1, B2, and B3, as well as vitamin E [3]. Barley is highly sought after due to its numerous health benefits and bioactive compounds. Barley contains health promoting carbohydrate (65%–68%), protein (10%–17%), free lipids (2%–3%), glucans (4%–9%) and minerals (1.5%–2.5%). Furthermore, total dietary fibre ranged from 11% to 34%, with soluble dietary fibre accounting for 3% to 20% of the total [4]. Its straw contains approximately 33% cellulose, 20% hemicellulose, and 17% lignin in the dry matter [5]. It contains essential amino acids such as lysine, which aids in the development of muscle tissue in animals [6]. Barley eating is good for human health because it lowers blood cholesterol by attaching to bile acids and excreting them through the faeces. Bile acids are compounds, which are made by the liver from cholesterol and are utilised to breakdown fat. When they are discharged along with the fibre from barley, the liver is forced to produce new bile acids and use up more cholesterol, decreasing the quantity of cholesterol in circulation [7]. In addition to its fiber, barley is also a good source of niacin, a B vitamin that provides numerous protective actions against cardiovascular disease. Niacin can help reduce total cholesterol and lipoprotein (a) levels. Niacin may also help prevent free radicals from oxidizing LDL, which only becomes potentially harmful to blood vessel walls after oxidation. Lastly, niacin can help reduce platelet aggregation, the clumping together of platelets that can result in the formation of blood clots. Now, research suggests regular consumption of whole grains also reduces risk of type 2 diabetes, skin diseases, and some cancers [8].

BARLEY CLASSIFICATION

Barley is adaptable to various climates such as the Arctic regions and deserts but does not grow well in areas with high humidity. Barley is the hardiest of all cereal grains, its limit of cultivation extending farther north than any other, and at the same time, it can be profitably cultivated in sub-tropical countries. The inflorescence of barley is known as ear, head or spike. The spikelets are directly attached to the central axis, or rachis, which is the extension of the stem that supports the spike. There are three spikelets at each node, called triplets, alternating on opposite side of the spike. The sterile glumes in some varieties are also be awned. Awnless varieties are also known.

Differences in the fertility of the *Hordeum* specific spikelet triplet confer a unique row–type identity to barley spikes. One way to classify barley therefore is based on whether the spike bears two, four or six rows of grains [9]. The first type, *Hordeum vulgare* is the barley variety that is generally cultivated. This type is cultivated and forms two rowed or six rowed barley. The second type is two row barley or wild barley. This type used to be classified as *Hordeum distichum*. The number of rows per spike is determined by *Vrs1* and *Vrs4* mutations [10]. On the basis of lateral florets two rowed barley can be classified into Rudimentary and developed.

Another way to classify barley is to describe the awns covering the kernels [11]. In the barley germplasm database awns are described along the following morphology: Long awned, short awned, awnless, hooded, elevated hooded, subjacent hooded, long awned in central row, and awnletted or awnless in lateral

rows, short awned in central row, and awnletted or awnless in lateral rows, awnless or awnletted in central and lateral rows, elevated hoods in central row and awnless in lateral rows.

BARLEY DIVERSITY

Barley (*Hordeum vulgare* L.) is a major winter cereal that has been the subject of numerous genetic studies. Plant breeders use diversity to develop new and improved crop cultivars to address global challenges such as food security, sustainability, and climate change adaptation. Genetic diversity is defined as the presence of genetic characteristics in a crop and can be assessed by examining differences in DNA sequence in a population of individuals [12-15]. Whereas genetic variation is defined as the genetic differences among individuals for a specific characteristic and is observable traits within a population that give rise to phenotypic variates [16-18].

The greater a crop's ability to adjust to rapid environmental changes, the more genetic diversity it has [19]. Crop development success hinges on finding and incorporating genetic diversity from cultivated cultivars, landraces, wild and near relatives of cultivated cultivars, and other plant genetic sources. Genetic variety is declining as a result of constant crossing with elite genotypes, which may increase vulnerability to unfavourable climatic change and limit future improvement opportunities.

Finding new genetic variation improves both the quality and quantity of their products, as well as their reaction to climate change [20]. The foundation of genetic diversity study at any taxonomic level is morphological characterisation [21]. The presence of diversity suggests that barley germplasm has a lot of genetic variation [22]. Morphological features were important in genetic research because they were easy to identify and had a simple method of inheritance compared to quantitative traits.

The term “germplasm characterization” refers to the process of identifying and describing differences between accessions. Barley can be regarded a model species because of its capacity to grow in a variety of conditions. As a result of its ability to adapt to a variety of habitats and its ability to survive in difficult conditions, it has amassed a large gene pool [23]. Food productivity must be raised to fulfil rising global food demands in a changing climate. Over the last decade, genetic diversity surveys in wild and cultivated barley have generated a large amount of data [24-38].

Genetic diversity studies are important techniques that aid crop improvement by identifying varied parents for breeding programmes, conserving genetic resources, and preventing undesirable genes from being introduced into elite germplasm [39]. It gives data on resource allocation and how it affects the long term preservation of various germplasm collections [40]. Genetic diversity refers to the presence of genetic heterogeneity among individuals of a variety or population, as evidenced by variances in DNA sequence, biochemical traits, physiological attributes, or physical characteristics. Because population structure can lead to misleading connections, diversity and genetic structure are important for association mapping. A control can be employed to limit false positives [41]. Morphological trait analysis can be used to estimate genetic diversity. It's utilised for indirect selection, which results in novel cultivars with great yield stability and higher performance in dry climates [42].

To investigate the development of SSR markers in barley, a number of morphological and molecular studies have been conducted. Because of their abundance, simplicity, cost effectiveness, codominance, and high polymorphism, Simple Sequence Repeats are the marker of choice for diversity evaluation [43-44]. SSR markers are a useful technique for determining variety. A large barley gene pool can be extremely beneficial in addressing present and future issues in barley production. Molecular markers are a core technique of current genetic research, including linkage analysis and association mapping, and have been widely utilised to compare the genetic differences across individuals. Traditional molecular markers, array based molecular markers, and sequencing based molecular markers are the three types of molecular markers available in barley. Restriction Fragment Length Polymorphism (RFLP) and Simple Sequence Repeats (SSR) are two common molecular markers.

In barley, RFLP was the first generation of molecular markers used to create genetic maps [45]. Hybridization and isotope labelling are required for RFLP genotyping, which is time consuming and hazardous to the operators' health. SSR markers are more sensitive than RFLP markers, and because they are PCR based, they are reasonably simple to employ. Three sets of barley SSR markers were created utilising a novel procedure that included creating a small insert genomic DNA library, hybridization with tandemly repeated oligonucleotides, and sequencing of positive clones [46-48]. With the growing number of sequences in public databases, particularly expressed sequence tags (EST), software was developed to predict SSR motifs for the construction of SSR markers in barley [49]. A barley SSR consensus map of 775 markers was created in 2007 by combining SSR markers from six genetic maps [50]. However, genotyping with SSR markers is limited due to its low density, labor intensive nature, and time consuming nature, making it unable to meet the demand for high density and throughput when genotyping large numbers of people.

BRIEF WORK DONE BY DIFFERENT WORKERS

Some of the brief work done by different workers is given below [51] used 40 SSRs to examine genetic similarities in a group of 107 naked barley genotypes from the Annapurna, Manaslu, and Lamtang Himalaya ranges in central Nepal, as well as 8 selected German and Canadian cultivars. There were 258 alleles found in total, with an average of 6.45 alleles per locus ranging from 2 to 17. On average, 4.8 alleles were discovered in Nepalese barley, with an estimated diversity index of 0.52 and an overall diversity index of 0.53. UPGMA clustering based on Dice Similarity was used to differentiate Nepalese landraces from European and Canadian cultivars. The Nepalese naked barley landraces collection is grouped into two distinct clusters, sub-clusters indicates that SSR markers revealed considerable genetic diversity.

The Shannon diversity index was used to determine phenotypic variety, and a high phenotypic diversity index (0.79) was found. Physical variables of the farm (land fragmentation index, farm size), agroclimatic features of the site (height, rainfall, temperature), and household characteristics all had a substantial and beneficial impact on barley diversity and area allocation, according to censored regression.

Using 18 simple sequence repeats, evaluated six Tunisian barley varieties (Faz, Manel, Martin, Rihane, Roho, and Tej) as well as six landraces from various growing regions in Tunisia (Djerba, Gabes, Jendouba, Kairouan, Kebili, Kerkennah) (SSRs). Only 11 of the 18 SSR markers showed polymorphism, yielding a total of 31 alleles. The number of alleles per locus ranged from 2 to 5, with an average of 2.81. The average PIC value was 0.50, with values ranging from 0.28 to 0.60. According to the number of rows per spike, the genotypes were divided into two primary groups by the UPGMA cluster analysis.

Abebe used a set of 199 barley germplasm accessions for evaluation of nine agronomic traits at Ethiopia's Holetta and Bekoji Agricultural Research Centers during the 2006 main cropping season, using non-replicated augmented design plots consisting of four incomplete blocks collected from ten Ethiopian administrative regions and four released cultivars for estimation of error variance. Depending on the variables involved, the genotype variance estimate of areas and elevations revealed significant heterogeneity among accessions. The fact that each location has a lot of genetic variation indicated how important it will be to gather germplasm in the future.

Wang used 35 simple sequence repeat (SSR) markers, five from each of the seven linkage groups with known map locations, to examine genetic diversity and relationships among 40 elite barley varieties. The SSR primers were highly polymorphic, detecting a total of 85 alleles with a range of 1 to 5 alleles per locus and an average of 2.4 alleles per locus. Based on the genetic similarity coefficients, the 40 elite barley types were divided into two groups. Seven malting barley varieties from China were included in one subgroup, and genetic diversity was lower than in other barley germplasm sources.

Park revealed genetic diversity in 737 barley landraces from Korean, Chinese and Japanese populations and evaluate the discrimination ability of 7 SSR markers, distributed uniformly throughout the barley genome. The number of alleles detected per locus was 8.9, 8.6 and 6.4 in Chinese landraces, Korean and Japanese respectively. The largest genetic distance ($D=1.209$) was found between Korean and Japanese populations and average value of genetic distance was $D=0.935$. The scatter plot overlapping in the central part amongst 3 groups of barley landraces and four major groups are formed on the basis of UPGMA.

Bolouri-Moghadam used 10 microsatellite markers to examine the genetic diversity of 7 cultivars of cultivated barley populations. There were 65 alleles in all, ranging in number from 7 to 13, with an average of 9.2 alleles per locus. The average polymorphic information content was 0.84, with a range of 0.80 to 0.88. SSR markers have a strong ability to provide most of the information in a single locus, as shown by the dendrogram that separated all of the barley genotypes into seven groups.

Chen characterized 115 barley germplasms, including 112 landraces and three new barley cultivars grown in the Shanghai region. They used a set of 11 SSR markers and detected a total of 66 alleles. The number of alleles ranged from 3 to 10, with an average of 6 alleles per locus and PIC values ranged from 0.568 to 0.853 with an average of 0.732. Clustering analysis divided the 115 barley accessions into two major categories in which category A contains 96 six rowed barley accessions and category B contains 16 two rowed barley accessions.

Using 17 microsatellite markers, Khodayari investigated the ge-

netic diversity of 32 individuals of two rowed and six rowed Iranian landraces barley. The number of alleles found ranged from 2 to 16, with an average of 8.11 per locus. With an average of 0.651 and a range of 0.058 to 0.921, a high amount of polymorphism was discovered. Based on SSR data, the two groups of cultivars (var. distichon and var. hexastichon) were split in dendrograms.

Naceur used 11 SSR markers from seven linkage groups to examine 31 barley accessions from North Africa (Algeria, Tunisia, and Egypt). The 11 SSR markers scored a total of 478 bands, with an average of 2.13 alleles per locus. The barley accessions are grouped by their eco-geographical origin, pedigree, agronomic characteristics, or caryopsis character (hulled or naked caryopsis).

Monawekh used 32 SSR markers to assess genetic diversity in 55 barley genotypes. By using 27 markers, they were able to create a recognisable pattern with high polymorphism of 203 alleles, and 5 markers revealed monomorphic bands. The number of alleles per locus ranged from 2 to 36, with an average of 7.52 and a PIC value of 0.65. The mean value of gene diversity was 0.70, with values ranging from 0.31 to 0.96. All of the genotypes were clustered individually in two primary groups according to the lineage, with substantial degrees of variation among genotypes.

Using 68 SSR primer pairs, Gougerdchi assessed the genetic diversity of 52 barley lines. Only 47 primer pairs out of 68 SSRs exhibited a distinct polymorphic banding pattern, averaging 3.26 alleles per locus and ranged from 2 to 9. PIC (Polymorphic Information Content) was 0.45, with a range of 0.07 to 0.81. Cluster analysis was used to separate barley lines into two groups.

Sipahia used 16 SSR markers spread throughout the seven barley linkage groups to study 84 barley landrace accessions from twelve nations. There were 92 alleles found in all, ranging from 1 to 7 per locus with an average of 5.75. Landraces from Germany, the Netherlands, Russia, Turkey, and the United States had the highest polymorphic loci (100%), while England had the lowest (75%). The landraces from Turkey had the most genetic variety, while those from Ukraine had the least.

Yadav used a biometrical approach, 19 morphological markers, and 47 microsatellite markers to evaluate 10 barley (*Hordeum Vulgare L.*) cultivars. There were 166 alleles found, ranging in number from 2 to 7, with an average of 3.25 alleles per locus. The genetic diversity was calculated using the Euclidean 2 distance and UPGMA methods

Using 37 SSR markers, investigated the morphological and genetic variability of 24 barley accessions from ICARDA, Lebanon, cultivated in Indian conditions at ICARDA-IRP (Indian Research Platform), Amlaha, (M.P.). They found 94 alleles in all, with an average of 2.54 alleles per locus ranging from 2 to 4. The average PIC value was 0.50, with values ranging from 0.153 to 0.707.

Yadav assessed genetic diversity in 96 germplasm lines using 15 random g-SSRs and 10 candidate gene based SSRs. The g-SSR markers' average PIC was 0.40, and the average number of alleles was 3.5, ranging from 5 to 2. The average genetic diversity was 0.37, with a range of 0.89 to 0.02.

Elakhdar estimated genetic diversity, allelic variation and population structure of 134 Egyptian barley genotypes collected from a different region along with 19 cultivated genotypes

procured from the Egyptian Agricultural Research Center using 261 informative SSR and SNP. A total of 261 alleles were detected with a mean of 4.08 alleles/locus and PIC was 0.49. Genetic diversity ranges from 0.03 to 0.82.

Kumar investigated 48 ICARDA barley accessions utilising 51 polymorphic SSR markers. PIC values varied from 0.150 to 0.781, with a mean of 0.491. A Neighbour Joining (NJ) tree was constructed using this matrix, and it revealed two primary clusters and three subclusters. The results of Principal coordinate analysis matched the results of the NJ tree perfectly.

Marzougui used 26 SSR markers spread across the seven chromosomes of barley to assess the genetic variation of 32 Tunisian barley accessions (*Hordeum vulgare L.*). There were 89 alleles found, ranging in number from 2 to 5, with an average of 3.4 alleles per locus. The average PIC value was 0.45, with values ranging from 0.88 to 0.70. Based on genetic similarity, cluster analysis was performed, and barley was divided into five groups. The results of cluster separation were mainly compatible with PCoA.

DISCUSSION

Khalil used 14 double primer markers to study genetic variation in seven varieties, resulting in 42 alleles with an average of three alleles per primer. The total number of alleles ranged from 1 to 7. Jan 2021 phenotyped 105 barley genotypes, including 38 two row genotypes and 67 six row genotypes, for 10 essential quantitative parameters. The examination of pre-harvest sprouting tolerance (PHST), growth, yield, and yield contributing factors indicated significant diversity in the germplasm, resulting in the identification of potential candidate genotypes for all parameters. In addition, 14 unlinked SSR markers were used to characterise a group of 96 barley genotypes (7 random and 7 genic SSR markers). SSR marker data analysis indicated a total of 67 alleles (range 2 to 8) with an average of 4.78 alleles per locus. Based on genotypic data, the clustering of 96 genotypes classified all of the genotypes into three broad clusters, sub-clusters, and sub sub clusters. The diverse and most promising genotypes discovered in this study for many attributes could be valuable in future barley breeding initiatives around the world.

CONCLUSION

The barley as a whole is reported to exert its positive effects on disease prevention, indicating a likelihood of synergistic interactions between the compounds present in it. In addition, despite the already reported favorable effects of barley on diabetes, hypertension, cardiovascular disease and lowering the cholesterol. It promotes intestinal movements relieving constipation, cleansing colonic harmful bacteria and reduced incidence of colonic cancer. Thus, research is needed to assess the health effects of human consumption of barley and barley products including germinated barley foodstuff, barley co-products and barley Nutrim. Genetic diversity in barley germplasm must be studied at the molecular level in order to maximise the use of barley genotypes in breeding programmes and to preserve barley genetic variation. Molecular marker could be an auxiliary selection mean for breeding new cultivars or lines, but its application in barley is at the stage of exploration. Therefore, it is necessary to deepen the research of molecular

marker techniques in barley breeding.

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