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## **Barley (*Hordeum vulgare* L.) Improvement Past, Present and Future**

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### **Abstract**

Barley has been cultivated for more than 10,000 years. Barley improvement studies always have the privilege of the breeders and scientists. This review is expected to provide a resource for researchers interested in barley improvement research in terms of mutation breeding, tissue culture, gene transfers, gene editing, molecular markers, transposons, epigenetic, genomic studies and system biology. We aimed to discuss some important and/or recent studies and improvements about barley for understanding the factors responsible for converting barley plants into the superior cereals, which occurred through gene transfers, gene editing and molecular breeding, which is important and could help us enhance the current pool of cultivated barley species to provide enough material for the future.

**Keywords:** barley improvement, *Hordeum vulgare* L., genetic research, genomics research, complex trait

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### **1. Introduction**

Cultivated barley (*Hordeum vulgare* L.) is the fourth important annual cereal crop from the family of Poaceae after wheat, rice, maize and is consumed as feed for livestock and, food—either pure or combined with other cereals in the form of porridge, sattu (roasted barley), breakfast foods and chapattis [1] and, most importantly, is also used for brewing malts. Barley, which is also an excellent model plant for biochemists, physiologists, geneticists and molecular biologists, is one of the world's earliest domesticated and most important crop plants [2]. According to world statistics, its production in 2015 was 148.78 million tons, where Turkey's contribution was 4,750,000 metric tons [3]. Barley is a self-pollinating diploid with  $2n = 2x = 14$  chromosomes. Moreover, it has two-rowed and six-rowed types, according to spike morphology [4]. The barley genome project is completed by the International Barley

Genome Sequencing Consortium [5]. It has 26,159 genes and large haploid genome of 5.1 gigabases (Gb), approximately 84% of the genome is comprised of mobile elements or other repeated structures. Ease of growth under laboratory conditions, and tissue cultures facilitate the development of gene transfer and gene editing technologies, although research on barley genome and system biology is progressing.

Barley has been cultivated for more than 10,000 years [6]. In former times, the Sumerian and Babylonian cultures utilized barley grains as currency. Barley improvement studies always have the privilege of the breeders and scientists. Barley is a short season, early maturing grain with a high-yield potential, and may be found on the fringes of agriculture in widely varying environments, often on the fringes of deserts and steppes or at high elevations in the tropics, receiving modest or no inputs [7]. Wide genetic variation of barley has generated cultivars that are tolerant to stress environments such as cold, salinity, drought and alkaline soil [8]. It is possible to cultivate barley in extensive ecological range. This adaptive genetic diversity against abiotic and biotic stresses indicates the potential of barley to develop stress resistant cultivars. The main objective of barley breeding programmes is enriching yield and grain quality. Improvement studies are also based on producing varieties resistant to biotic (pathogens, fungal, viral and other organisms) and abiotic stresses (e.g. drought, salt, cold and heat) [9]. Identifying and understanding the genetics basis of stress tolerance mechanisms in crops is fundamental to develop new varieties with more stress tolerant characters [10].

Barley is an economically important crop plant, the fourth cereal worldwide in terms of the planting area, utilized almost 60% as animal feed, around 30% for malt production, 7% for seed production and only 3% for human food [11, 12]. In recent years, the malt derived from the germinated barley is the key material for the malting which represents the most economically favourable application for beer brewing [13]. However, to enhance the germination and malting quality of barley, addition of malting additives during the malting is strictly controlled cause of food safety and environmental pollution. Improvement of barley cultivars for the malting may be the most economical approach to improve malt quality. As a result, identifying and understanding the genetics basis of barley is fundamental to develop new varieties with more properties [14]. Also nowadays, barley has numerous advantages in food industry due to its high content of bioactive compounds such as  $\beta$ -D-glucan, tocopherols, tocotrienols and phenolics such as benzoic and cinnamic acid derivatives, proanthocyanidins, quinones, flavonols, chalcones and flavones [15, 16]. The studies showed that  $\beta$ -D-glucan is regarded as a significant function of preventing various diseases such as diabetes, cardiovascular diseases, hypertension and others [17].

Barley is one of the most genetically diverse cereals which is categorized as spring or winter types, two-rowed six row, hulled or hullless by the presence or absence of hull tightly adhering to the grain, and malting or feed by end-use type. Therefore, breeding programmes depend on high level of genetic diversity which provides a significant opportunity for achieving progress. Specific traits may be introgressed in back-crossing studies by hybridisations between high-yielding cultivars and wild barley in conventional breeding programmes [18]. However, mutation breeding is also important for widening variation to develop new cultivars. Herman Nilsson-Ehle and Ake Gustafsson, and even L. J. Stadler have performed induced mutation studies on barley, and then Stadler have published his data in 1928. In 1953,

the 'Group for theoretical and applied mutation research' was established by the Swedish Government. The aim of their study was the investigation of basic research problems in order to effect and improve methods for breeding programmes [19]. Both radiation and chemical mutagenesis have been separately used to increase the numbers of barley cultivars which may have desirable traits. 'Golden promise', which is the most popular malting barley, was produced by radiation mutagenesis [20]. In Turkey, mutation breeding programme has been started by Bilge et al. with collaboration of Agricultural Research Institutes [21, 22]. They treated barley seeds with radiations (X and gamma rays) and chemical (ethyl alcohol, streptomycin, terramycin, penicillin G, sodium cyanide and ethyl methane sulfonate solutions) mutagens and observed different traits such as chlorophyll deficiency, large-eared, high-yielding, thick-stemmed, dwarf and early-heading in M1. Today, use of mutation breeding generally continuing at targeted level will be discussed by new technologies.

In this review, we summarize the history of barley improvement research in terms of mutation breeding, tissue culture, gene transfers, gene editing, molecular markers, transposons, epigenetic, genomic studies and system biology. We aimed to discuss some important and/or recent studies and improvements about barley for understanding the factors responsible for converting barley plants into the superior cereals, which occurred through gene transfers, gene editing and molecular breeding, which is important and could help us enhance the current pool of cultivated barley species to provide enough material for the future.

## 2. Barley molecular markers

Plant breeders have been used with phenotypic traits for selection of desirable traits due to habits, disease resistance, yield or quality to develop new cultivars. Two major strategies have been utilized to select desirable traits which are classical breeding and molecular breeding. The development and use of molecular markers for the detection and exploitation of polymorphism have been playing a significant role in plant breeding studies. Molecular plant breeding utilizes two major approaches, marker-assisted selection (MAS) and genetic transformation, to produce new varieties with desirable characteristics [23, 24]. MAS is a process that uses molecular markers to increase crop yield, quality and tolerance to biotic or abiotic stresses [25]. The choice of marker systems is a significant part of plant breeding cause of the requirements according to the conditions and resources. In the last two decades, molecular markers such as restricted fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLPs), simple sequence repeats(SSR), inter-simple sequence repeats (ISSR), expressed sequence tags (ESTs) and single-nucleotide polymorphisms (SNPs), transposon-based markers (IRAP, iPBS) have been used as genetic markers for measuring the genetic differences existing in the genomes [26–30]. Development of next-generation sequencing technologies opened new opportunities for the development of sequence-based markers. Today, we have new markers which are not fragment-based but are sequence-based. Medium and high density arrays are available for barley. The choice of utilized marker methods has shifted from the first and second generation markers such as RFLPs, RAPDs, microsatellite and AFLPs to third and fourth generation markers including DArTs, TAMs, RADs and CNVs/PAVs which are demonstrated in **Table 1**.

Marker types used	Aim	Results	Reference
RFLP	Construction of an RFLP map of barley	Genetic and physical mapping achieved	[37, 38]
RAPD	Analyses genetic variations in barley	Cultivar certification achieved	[56]
	Cultivar discrimination	Cultivar and hybrid certification achieved	[57]
AFLP	AFLP markers linked to water stress tolerant and sensitive bulbs of barley	AFLP markers was identified in two barley genotypes (tolerant and sensitive)	[41]
SSR	Construction of a SSR consensus map of barley	149 mapped SSR markers on Australian varieties are presented in the form of a consensus map, SSRs proved to be adaptable to several technologies	[44, 45]
IRAP and iPBS	Callus age and retrotransposon	Tissue culture conditions and callus age affected <i>Sukkula</i> retrotransposon movements, and all individuals did not present the same effect	[51]
SNP	Utilization of the BOPA1 assay to explore SNPs in geographically matched landraces and wild accessions collected	Of the 1536 SNPs represented on BOPA1, 1301 mapped SNPs	[58]
CAPS	Comparison of SNP and CAPS markers application in genetic research in wheat and barley	Results supporting the development of different strategies for the application of effective SNP and CAPS markers in wheat and barley	[59]
CNV	The prevalence of copy number variation (CNV) and its role in phenotypic variation in domesticated barley cultivars and wild barleys	Levels of CNV in the wild accessions were found to be higher than cultivated barley. CNVs are enriched near the ends of all chromosomes except 4H. CNV affects 9.5% of the coding sequences represented on the array and the genes affected by CNV	[55]
cDNA-AFLP	Development of molecular markers linked to barley heterosis	Five transcript-derived fragments (TDFs) showed significant effects on heterosis	[60]
DArT	94 Czech malting barley cultivars identification	DArT-based dendrogram was established	[61]
	Genome-wide association studies of agronomic and quality traits in a set of German winter barley ( <i>Hordeum vulgare</i> L.) cultivars using DArT	A set of about 100 winter barley ( <i>Hordeum vulgare</i> L.) cultivars, comprising diverse and economically important German barley elite germplasm was analysed	[62]
	QTL loci effecting kernel length	LEN-3H and LEN-4H could be used for improve kernel length	[63]
SLAF-seq and whole-genome shotgun	semi-dwarf gene <i>ari-e</i> from Golden Promise	Specific-length amplified fragment sequencing (SLAF-seq) with bulked Segregant analysis (BSA) to develop SNP markers, and (2) the whole-genome shotgun sequence to develop InDels. Both SNP and InDel markers were developed in the target region	[64]
Restriction site associated DNA (RAD) sequencing	SNP-based high density genetic map and mapping of <i>btwd1</i> dwarfing gene in barley	The SNP-based high-density genetic map developed and the dwarfing gene <i>btwd1</i> mapped	[65]

Marker types used	Aim	Results	Reference
InDel markers	Development of InDel markers	High-density InDel markers with specific genome locations were developed with 6976 molecular markers (SSRs, DArTs, SNPs and InDels) integrated into single barley genetic map	[66]

**Table 1.** Molecular markers used in barley research.

[31–34]. Next-generation breeding technologies are now effectively used for the establishment of genotypic and phenotypic relations [35]. Future barley varieties are designed with crop model ensemble [36].

Restriction fragment length polymorphism (RFLP) marker system has been used as a measure of genetic diversity for mapping studies in barley [37, 38]. Genetic relationships among 21 barley accessions (17 of *H. bulbosum* L. and 4 of *H. vulgare* L.) have been investigated by Okumus and Uzun [39] have successfully produced 111 RAPD markers. Combination of bulked segregant analysis and RAPD primers has been used to identify molecular markers linked to crown rust resistance gene *Rpc1* in barley [40]. Another molecular marker technique AFLP has been utilized for linkage studies and evolution of barley [41–43]. 149 simple sequence repeats (SSRs) or microsatellite markers have been constructed in the form of a consensus map by using 12 barley populations [44, 45]. SSR markers have been utilized for the selection of *Rym4/Rym5* locus conferring resistance to the barley mosaic virus complex in barley. The polymorphic SSR marker QLB1 was found to be co-segregated with *Rym4/Rym5* locus which also used to develop for the high-resolution map [46]. Other marker methods used in plant breeding are transposable elements-based marker systems such as inter-retrotransposon amplified polymorphism (IRAP), retrotransposon-microsatellite amplified polymorphism (REMAP) and inter-primer binding site amplification (iPBS) to identify retrotransposon markers linked to traits. Our group has been using IRAP and iPBS marker techniques to determine retrotransposon insertion patterns, movements of transposons, somaclonal variations, and callus aging. Our results showed that callus culture conditions have activated *BARE-1* and *Nikita* elements [47–50]. Movements of the non-autonomous retrotransposon *Sukkula* were investigated by Kartal-Alacam et al. [51] in barley. Recently, IRAP technique is also utilized to assess the genotoxicity of some drugs such as epirubicin [52] and amiprofophos-methyl [53].

Genome- and chromosomal-level genetic structures are really important for the investigation of the evolution, adaptation and spread of the crops. Therefore, single-nucleotide polymorphism (SNP) platforms, which are used to assess the evolution of barley, are a key tool in the development of farming. Russell et al. [54] utilized the barley oligonucleotide pool assay 1 platform (BOPA1, composed of 1536 SNPs) to compare 448 accessions genome-level genetic structures, 317 of landrace material and 131 of wild barley, and observed that significant chromosome-level differences diversity between landrace and wild barley types was around genes known to be involved in the evolution of cultivars. Fourteen barley genotypes (eight cultivars

and six wild barleys) have been utilized to explore copy number variations (CNV) by using comparative genomic hybridization. The study showed that CNVs were enriched near the ends of all chromosomes except 4H and affected 9.5% of the coding sequences represented on the array [55].

### 3. Barley tissue cultures and gene transfers systems

Plant tissue culture, which provides convenience for plant propagation and manipulation, is based on growing plant cells, tissues or organs isolated from the mother plant, on artificial media [67]. It is required to regenerate *in vitro* whole transgenic plants by using cells, tissues or a single cell cultured on a nutrient medium in a sterile environment [68]. Regeneration ability in barley depends on the donor plant material, genotype, media and environment [69–71]. One significant limitation of barley transformation is still the poor regeneration potential of modern cultivars. However, several studies have been conducted to improve tissue culture techniques to increase regeneration rates [72]. From past to today, various tissue culture protocols have been developed by using immature embryos [73–80], mature embryos [81–87], apical meristems [88–90], anthers [91–94], microspores [95–97], ovaries [98, 99], cell suspensions [100–104], protoplasts [105], coleoptile tissue [106] and leaf base segments [90, 107].

The improvement of barley through genetic transformation and *in vitro* methods requires the development of reliable, efficient and reproducible plant regeneration systems (**Table 2**) [70, 108, 109]. The plant regeneration capacity is affected by the genotype of donor plants, growth characteristics of induced calluses, the composition of the media, including growth regulators [110, 111]. Tissue cultures of barley are mainly based on the optimization of callus induction [112], regeneration [71, 113] and transformation [99], understanding of tissue culture response [114], detection, evaluation and elimination of somaclonal variation [81, 94, 115–118]. The use of mature embryos has a great advantage compared to other systems such as protoplast and cell suspensions. For barley tissue culture, mature embryos represent ideal system because of higher germination and regeneration rates by somatic embryogenesis from cultured mature embryos of barley [87]. Phytohormones are also crucial to setting optimal tissue culture conditions to produce undifferentiated callus tissue from differentiated tissues such as an embryo [119].

Callus formation, which is a dedifferentiation of single cells or tissue explants, offers the great opportunity for investigation of *in vitro* selection production of genetic variations [120–124]. The regeneration of plants from callus of barley has a great potential to produce new lines in breeding improved barley cultivars [125, 126]. The type of auxin, 2,4-dichlorophenoxyacetic acid (2,4-D), is the most used growth regulator for callus induction [123, 127, 128]. 2,4-D have been utilized to induce embryogenic callus together with or without cytokinins such as zeatin or 6-benzylaminopurine (6-BAP). Moreover, the influences of 2,4-D, Dicamba (3,6-dichloro-O-anisic acid), Picloram (4-amino-3,5,6-trichloropicolinic acid) or 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) have been investigated on the induction of embryogenic callus. It was found that Dicamba significantly increased the regeneration through somatic embryogenesis [78, 111, 129–131]. However, callus quality depends

Culture type	Aim	Results	Reference
Immature embryos	Tissue culture and plant regeneration from immature embryo explants	Regeneration of plantlets was obtained for 19 of the 20 genotypes approximately 4 months after culture initiation	[75]
	Evaluation 9 barley cultivar for <i>in vitro</i> culture response	For each character, there were significant differences between genotypes, between 2,4-D concentrations and also significant genotype × medium interactions	[136]
	Evaluation of 10 Canadian barley genotypes for <i>in vitro</i> culture response	Fertile plants were regenerated	[137]
	Callus induction and regeneration at Czech cultivars	The callus formation frequency and number of green regenerants were influenced significantly both by genotype and auxin	[123]
	Callus induction and regeneration at Nordic cultivars	Regeneration of many plants from the same callus over long periods of time and makes available highly efficient regeneration protocols	[138]
Mature embryos	Tissue culture establishment and plantlet regeneration	Plantlets regenerated both via organogenesis and somatic embryogenesis	[139]
	Tissue culture and plant regeneration at Indian cultivars	Multiple shoot induction and plantlet regeneration in Indian cultivar of barley	[140]
Anther culture	Possible effect of copper during anther culture in barley	The positive influence of copper sulphate was characterized by an increase of microspore survival during anther culture	[141]

**Table 2.** Tissue culture and plant regeneration studies in barley.

on barley genotypes [125, 132]. And also, it has been reported that the most barley cultivars produced friable and translucent callus [122, 125].

Somatic embryogenesis, which is defined as a process by which haploid or diploid somatic cells develop into structure that resembles zygotic embryo, is an important tool for large scale vegetative propagation. Somatic embryos are bipolar structures without any vascular connection with the parental tissue and these structures can differentiate either directly from the explants without an intervening callus phase or indirectly after a callus phase. Immature embryos have a great potential to produce somatic embryos through embryogenic callus [133]. Marthe et al. [134] have investigated transformation efficiency for more than 20 barley cultivars by using immature barley embryos, and they found that the transformation efficiency of cv 'Golden Promise' was still higher than any other cultivar tested. Another study conducted by Hisano et al. [135] showed that callus derived from immature embryos of 'Golden Promise' had the highest ratio of regeneration of green shoots comparing with 'Haruna Nijo' and 'Morex'.

Since 1990s, genetic engineering of plants is a powerful research tool for gene discovery and function to investigate genetically that controlled traits have provided great opportunities to introduce agronomically useful traits. The first report on stable barley transformation via direct DNA-transfer methods has been established by Lazzeri et al. [105]. Tingay et al. [142] were

first reported *Agrobacterium*-mediated gene transfer protocol to barley using immature embryos (IEs). Since then, numerous protocols for barley transformation have been developed with the contribution of technical improvements based on immature embryos or androgenetic pollen cultures or isolated ovules as gene transfer targets [99, 143–146]. Gurel and Gozukirmizi [147] optimized the transformation parameters for efficient and successful genetic transformation of mature barley embryos. They defined the optimal combination of electroporation and electroporated mature embryos with  $\beta$ -glucuronidase (*Gus*) and neomycin phosphotransferase II (*nptII*) genes. The frequency of transformants was generally not very high between 1.7 and 7.0% of the immature embryos infected via *Agrobacterium*. On the other hand, it has been recently reported that the frequency is around 25% or higher [148–150]. Although the transformation frequency is lower, immature embryos still remain the target tissue of choice in barley [148, 151].

The most of barley transformation studies have been performed to confer biotic (fungal and viral resistance) and abiotic (herbicide, drought and salinity, etc.) resistance, to facilitate brewing and digestibility, to alter protein composition and for molecular pharming [152]. Some of those methods have been established in **Table 3**. Yeo et al. [153] developed

Gene transfer type	Aim	Results	References
Biolistic transformation system	Target tissues such as immature embryos, embryos derived callus and microspore derived callus	Successful transformation	[156]
	Immature embryos and microspore-derived cultures	Successful transformation	[157]
	Transformation of recalcitrant species	Successful transformation	[158]
	Pre cultured immature embryos	Molecular analysis of T1 generation plantlets revealed the amplification of selectable marker <i>hptII</i> gene in the progeny	[159]
<i>Agrobacterium</i> -mediated transformation	Immature embryos	Successful transformation	[142]
	Shoot apices	Successful transformation	[160]
	Optimization of gene transfer immature embryos	Transformation efficiencies 2.6–6.7%	[145]
	Young ovules	Successful transformation	[161]
	Microspores	Successful transformation	[147]
	Optimization of gene transfer immature embryos	25 % transformation efficiency	[148]
	Mature scutellum	Successful transformation	[162]
	Immature embryo-derived callus cultures	Improve T-DNA transfer in monocotyledon transformation procedures	[163]
	Mature embryos	Successful transformation	[164]
Tissue electroporation	DNA transfer into mature embryos of barley via electroporation	Successful transformation	[147]

**Table 3.** Gene transfer research on barley.

'Golden SusPtrit' which is a barley line combining SusPtrit's high susceptibility to non-adapted rust fungi with the high amenability of Golden Promise. They generated a double haploid (DH) mapping population (n=122) by crossing SusPtrit with Golden Promise to develop the 'Golden SusPtrit'. SG062N was found the most efficiently transformed DH line with 11–17 transformants per 100 immature embryos. To protect barley from the effects of stress-produced reactive carbonyls, which is accumulated by reactive oxygen species in the plant cells, an *Agrobacterium*-mediated transformation was carried out using the *Medicago sativa* al dose reductase (MsALR) gene by Nagy et al. [154]. Their results demonstrated that this technique could be applied for the detection of cellular stress, and also found that targeting of MsALR into the chloroplast has also resulted in increased stress tolerance. In addition to these studies, Han et al. [155] reported that a construct containing full-length of HvGlb2 cDNA encoding barley (1,3;1,4)- $\beta$ -glucanase isoenzymes EII under the control of a promoter of barley D-Hordein gene Hor3-1 was introduced into barley cultivar Golden Promise via *Agrobacterium*-mediated transformation. High content of (1,3;1,4)- $\beta$ -D-glucan of barley grains is considered as an undesirable factor effecting malting potential, brewing yield and feed utilization. They showed that over-expression of (1,3;1,4)- $\beta$ -glucanase led to an increase in the thousand grain weight. Also, manipulating expression of (1,3;1,4)- $\beta$ -glucanase EII could control the  $\beta$ -glucan content in grain with no apparent harmful effects on grain quality.

#### 4. Genomic studies on barley

The genetic revolution of the past decade has greatly improved our understanding of the relationships between genetic and phenotypic diversity with a resolution that has never been reached before. The development of next generation sequencing (NGS) technologies has increased accuracy and decreased costs. Sequencing or re-sequencing of reference genomes and also new varieties allow the identification of numerous numbers of markers, allelic diversities and have changed our insight of genome organization and evolution. The sequencing of crop genomes provided evidences for plant origin and evolution; genome duplications, re-arrangements; adaptations and functional modulations [165]. The full genome sequence is essential to provide knowledge for understanding natural genetic variations and development for breeding programs.

Recently, novel high-throughput sequencing strategies have revealed the structure of barley genome [166, 167]. Existence of 26,159 barley genes was confirmed by a systematic synteny analysis with model species from the Poaceae family (rice, maize, sorghum and *Brachypodium*) which have already had annotation of their genomes. Also, up to 80% of the 5.1 Gb genome of barley contains repetitive DNA, making the fully sequencing complicated [5]. Full annotations and a sequence-rich physical map of the barley genome, which is based on the genomic information contained in bacterial artificial chromosomes (BACs) developed for the Morex variety [168, 169], are available on public databases (<http://webblast.ipk-gatersleben.de/barley/index.php>) [167]. The first single nucleotide polymorphisms (SNPs) genotyping approach, based on the illumina oligo pool assays (OPAs), allowed the examination of 4596 markers in sets of 1536 SNPs [58]. Although declining cost of NGS technologies, thousands to million SNPs have been discovered via re-sequencing, providing greater detail for high density genetic maps [170].

Currently, array-based genotyping platform Infinium iSelect allows the simultaneous testing of 7842 SNPs [171]. Takahagi et al. [172], performed deep transcriptome sequencing, identified 38,729–79,949 SNPs in the 19 domesticated accessions and 55,403 SNPs in the wild barley. However, the complete sequences of the 525,599 bp mitochondrial genomes of wild and cultivated barley have been determined by Hisano et al. [135]. The mitochondrial genome of barley consists of 33 protein-coding genes, three ribosomal RNAs, 16 transfer RNAs, 188 new ORFs, six major repeat sequences and several types of transposable elements. The mitochondrial genomes of these wild and cultivated barley lines have been found to be almost identical in terms of both nucleotide sequence and genome structure, only three SNPs detected between haplotypes [135].

Several techniques, including linkage (or QTL mapping) mapping, association mapping (GWAS) and high-throughput omic techniques, such as transcriptomics, ionomics, proteomics and metabolomics analysis, have been used to identify a single gene or multi-genes corresponding to gene regulation networks of development, flowering, vernalization and biotic and abiotic stress conditions [173]. Next generation sequencing approaches (e.g. RNA-Seq) were carried out within 5 years and enlarged our knowledge about gene regulation networks of stress conditions. Especially, RNA-seq approach has been widely utilized due to low background noise, high sensitivity and reproducibility, great dynamic range of expression and base-pair resolution for transcription profiling [174]. Transcriptomic analyses of more than 28 plant species have revealed thousands of genes that are differentially regulated under drought stress conditions [175]. During last few years, an increasing number of these genes have been characterized and their function under drought conditions has been shown by the analysis of loss-of-function mutants or over expressing lines. Most of these functional characterization studies have been performed in the model species *Arabidopsis thaliana* and in the grass *Oryza sativa*. However, production of desired drought-tolerant crop species has required the identification of orthologous genes in each species. Transcriptome and whole-genome sequencing of different plant species lead to identify orthologous genes across several model and crop species [176].

Transcriptome profiling of barley under low nitrogen (LN) conditions have been determined by using RNA-seq approach. 1469 differentially expressed genes were identified between tolerant and sensitive barley varieties under LN. Differences between tolerant and sensitive genotypes involved transporters, transcription factors, kinases, antioxidant stress and hormone signalling related genes. However, DEGs were classified in amino acid metabolism, starch and sucrose metabolism, secondary metabolism [177]. Up to today, transcription dynamic of hullless barley grain development was not well understood. Tang et al. [178] have conducted comparative transcriptome approach to investigate changes during grain development. 38 DEGs were determined co-modulated in two barley landraces with the differential seed starch synthesis traits. The results showed that these 38 DEGs encoded proteins such as alpha-amylase-related proteins, lipid-transfer protein, homeodomain leucine zipper (HD-Zip), Nuclear Factor-Y, subunit B (NFYBs), as well as MYB transcription factors. Also, they found that two genes *Hvulgare\_GLEAN\_10012370* and *Hvulgare\_GLEAN\_10021199* encoding SuSy, AGPase (*Hvulgare\_GLEAN\_10033640* and *Hvulgare\_GLEAN\_10056301*), as well as SBE2b (*Hvulgare\_GLEAN\_10018352*) were significantly contributed to the regulatory mechanism during grain development in both genotypes.

Numerous numbers of studies have been performed to understand biotic and abiotic stress tolerance mechanisms. For this purpose, RNA-seq approach or microarray have a valuable potential to define stress mechanisms. One of the studies has been conducted by Tombuloglu

et al. [179] to discover the properties underlying the boron tolerance mechanism. By using transcriptome-wide approach, 256,847 unigenes were generated and, 16 and 17% of the transcripts were found to be differentially regulated in root and leaf tissues, respectively, according to gene expression analysis. Most of these unigenes were found to be involved in cell wall, stress response, membrane, protein kinase and transporter mechanisms [179]. Also, physiological and biochemical analysis have provided valuable insights towards a novel integrated molecular mechanism of stress tolerance mechanisms in barley. A genome-wide transcriptome analysis was performed to identify the mechanisms of cadmium (Cd) tolerance in two barley genotypes with distinct Cd tolerance by using microarray approach. Microarray expression profiling revealed that novel genes may play important roles in Cd tolerance which were mainly via producing protectants such as catalase against reactive oxygen species, Cd compartmentalization (e.g. phytochelatin-synthase and vacuolar ATPase) and defence response and DNA replication (e.g. chitinase and histones) [180]. Another study to understand abiotic stress was the sequencing of young leaves RNAs of wild barley treated with salt (500 mM NaCl) at four different time intervals. Differential expression profiles have been classified into nine clusters by two-dimensional hierarchical clustering. The most important groups were assigned to 'response to external stimulus' and 'electron-carrier activity' which means that the highly expressed transcripts are involved in several biological processes, including electron transport and exchanger mechanisms, flavonoid biosynthesis and reactive oxygen species (ROS) scavenging, ethylene production, signalling network and protein refolding [181]. Hulless barley, also called naked barley, often suffered from drought stress during growth and development. Therefore, Zeng et al. [182] have investigated co-regulated mRNAs expression patterns under early well water, later water deficit and finally water recovery treatments, and to identify mRNAs specific to water limiting conditions. The results showed that 853 DEGs were determined and categorized into nine clusters. The up-regulated genes were found to be relevant to abiotic stress responses in abscisic acid (ABA) dependent and independent signalling pathway, including NCED, PYR/PYL/RCAR, SnRK2, ABF, MYB/MYC, AP2/ERF family, LEA and DHN under low relative soil moisture content (RSMC) level. However, the transcriptome analysis revealed that the most affected genes were related to tetrapyrrole binding, photosystem and photosynthetic membrane under drought stress conditions.

The proteomic approach also plays significant roles to understand alterations in the context of physiological and morphological responses to biotic and abiotic stresses in barley. Rollins et al. [183] have investigated the proteins differentially regulated in response to drought, high temperature or a combination of both treatments by using differential gel electrophoresis and mass spectrometry. The study showed that the drought treatment induced strong reductions of biomass and yield, but not causing significant alterations in photosynthetic performance and the proteome. In contrast, the heat treatment and the combination of heat and drought caused the reduction of photosynthetic performance and changes of the leaf proteome. 14 proteins among 99 protein spots were identified as a genotype-specific manner in response to heat treatment. The analysis indicated that the differentially regulated proteins were related to photosynthesis, detoxification, energy metabolism and protein biosynthesis. Barley, also, used to identify the quantitative proteome changes under different drought conditions by Vitámvás et al. [184]. They cultivated plants for 10 days under different drought conditions that the soil water content was held at 65, 35 and 30% of soil water capacity (SWC), respectively. The proteomic alterations of barley crowns grown under

different drought conditions were determined utilizing two-dimensional difference gel electrophoresis (2D-DIGE). Analysis of 2D-DIGE revealed that 105 differentially abundant spots were detected between the controls and drought-treated plants. The identified proteins were classified into stress-associated proteins, amino acid metabolism, carbohydrate metabolism, as well as DNA and RNA regulation and processing.

## 5. Genome editing

Genome editing has recently emerged as a novel transgenic method to improve crop plants has great opportunities over conventional gene targeted techniques. The most important advantage of gene editing is the modification of the targeting specific genes *in situ*. Genome editing, uses 'programmable' nucleases such as zinc finger nucleases (ZFNs), TAL effectors nucleases (TALENs) or clustered regularly inter-spaced short palindromic repeat (CRISPR)-associated endonucleases, may also be used to introduce gene insertions, gene replacements, insertions or deletions at specific genomic locations [185]. These proteins have a recognition domain, is provided by the FokI domain in both ZFNs and TALENs, and Cas9 in CRISPR systems, can be engineered to target specific sequences. Genome editing is based on double-strand break (DSB) induction [186], and subsequent repaired by the cell's own non-homologous end-joining (NHEJ) or homologous recombination (HR) mechanisms [185]. Genome editing is a key tool for advancing knowledge of gene function as well as allowing targeted mutagenesis with high efficiency in plants, including barley [187, 188].

Wendt et al. [187] reported the assembly of several TALENs for a specific genomic locus in barley. They tested the cleavage activity of individual TALENs *in vivo* using a yeast-based, single-strand annealing assay, and then the most efficient TALEN have been selected for barley transformation. Cleavage of the non-specific target was not observed, but analysis of the resulting transformants demonstrated that TALEN-induced double strand breaks led to the introduction of short deletions at the target site. Another study with TALENs has been reported by Gurushidze et al. [188] that they used TALENs in pollen-derived, regenerable cells to establish the generation of instantly true-breeding mutant plants. A gfp-specific TALEN pair was expressed via *Agrobacterium*-mediated transformation in embryogenic pollen with 22% of the TALEN transgenics. During gene replacement, desired DNA could integrate into the genome by homologous recombination that provides great promise to the introduction of mutations at pre-determined positions in the genome. Watanabe et al. [189] used a model system based on double-strand break induction by the mega nuclease I-SceI to target specific position in the genome. They obtained two transformants that were stably inherited as a single Mendelian trait. They suggested that stable gene replacement could be achieved in barley for routine applications by targeted double-strand break induction. The RNA-guided Cas9 system also represents a flexible approach for gene editing in barley and provides a valuable tool to create specific mutations that knock-out or alters target gene function. Lawrenson et al. [190] investigated the use and target specificity of RNA-guided Cas9 genome editing in barley. They demonstrated Cas9-induced mutations in the first generation of 23% for barley line. And also, they observed that stable Cas9-induced mutations were transmitted to T<sub>2</sub> plants independently of the T-DNA construct thus establishing the potential for rapid characterisation of gene function in barley.

## 6. Transposons, epigenetic studies and non-coding RNAs

Transposons, is a segment of DNA moves to new location in a chromosome or to another chromosome or cell, were first identified in maize by McClintock [191]. Several studies have been revealed that transposons affect gene structure, epigenetic regulations and genome dynamics of almost all living organisms [30]. Transposons alter the existing genome structure that can lead to significant changes such as deletions and/or insertions. Percentages and types of transposons can vary among species [192] that prokaryotic genomes contain 1–3% transposons. However, their percentage may reach 85% or more in eukaryotic genomes, especially plants [193]. Due to having larger genome, barley has larger transposon-derived DNA content with up to 85% [194]. Also, it was demonstrated that *Copia* retrotransposons remained intact and active for much longer time periods in the larger genomes such as barley than the smaller genomes [195].

Our group has been studying barley transposon effects on somaclonal variation, stability of aging barley calli and callus regeneration by using IRAP markers derived from *BARE-1* [47, 50] and *Nikita* [48]. In addition, mature embryo, leaf, root tissues were investigated for *BARE-1* and *BAGY2* movements by Marakli et al. [49] and *Sukkula* movement in barley, which is a non-autonomous retrotransposon, have been investigated by our group [51]. We demonstrated that *BAGY2* was more stable than *BARE-1*. Another study on transposon movements of retrotransposons and methylation alteration was performed by Temel and Gozukirmizi [196]. We found that not all callus induction conditions increased the retrotransposon activity. However, increase in cytosine methylation has been observed during callus formation using Sensitive Restriction Fingerprinting. Yilmaz et al. [197] also investigated the stability of aging barley calli and regenerated plantlets from those calli. We used the *BAGY2* retrotransposon-specific IRAP technique to determine level of variations of DNA. We found that the culture conditions caused genetic variations, and also copy numbers of internal domains of *BAGY2* have increased. Moreover, IRAP technique has been utilized to assess the genotoxicity of some drugs such as epirubicin [52] and amiprophos-methyl [53]. Recently, Yuzbasioglu et al. [198] used IRAP markers to identify variation in single seed derived leaves and roots in rice.

Epigenetic chromatin modification is defined as heritable changes in gene expression which are not occurred by alterations in the nucleotide sequences of DNA. DNA methylation and modifications of covalent histone N-terminal tail are mainly regarded as chromatin modifications that can be changed in plants during the cell cycle [199, 200], plant development [201, 202] or in stress response [203]. The epigenetic mechanisms keep gene or genes active or repressive states [204, 205]. Braszewska-Zalewska and Hasterok [206] investigated the differences of epigenetic modification between root meristematic tissues of barley. Their study indicated that levels of epigenetic modifications varied between RAM tissues. Studies on environmental stresses showed that both DNA methylation and histone modifications are involved in DNA damage response. Also, Braszewska-Zalewska et al. [207] observed that chemical (maleic acid hydrazide; MH) and physical (gamma rays) mutagens strongly affected the level of histone methylation and acetylation. One of the major components of epigenetic variations is the combinations of histones carrying different covalent modifications that Baker et al. [208] have mapped nine modified histones in the barley seedling

epigenome using chromatin immune precipitation next-generation sequencing (ChIP-seq) technique. They defined 10 chromatin states (five states to genes and five states to intergenic regions) representing local epigenetic environments in the barley genome. Moreover, it was found that H3K36me3-containing two genic states were related to constitutive gene expression. However, one genic state involving an H3K27me3 was related to differentially expressed genes.

The recent wide applications of whole-genome tilling array and RNA-sequencing (RNA-seq) approaches have revealed that the transcription landscape in eukaryotes is much more complex than had been expected [209]. These approaches have facilitated the identification of thousands of novel ncRNAs (or npcRNAs) in many organisms, such as humans, animals and plants [210–214]. ncRNAs are classified as short (<200 nt) and long ncRNAs (lncRNAs; >200 nt). Transcriptional and post-transcriptional regulation of gene expression of short ncRNAs, including siRNAs, miRNAs and piRNAs, has been well recognized and the molecular mechanisms of short ncRNA-mediated regulation have been well understood [215, 216]. On the contrary, the regulatory roles of lncRNAs are only beginning to be recognized and the molecular basis of lncRNA-mediated gene regulation is still poorly understood [217]. Our group has been investigating the association between salinity stress metabolism and barley lncRNAs (unpublished data). Identification of novel lncRNAs is likely to provide new insight into the complicated gene regulatory network involving lncRNAs, provide novel diagnostic opportunities, and pinpoint novel therapeutically targets.

## 7. Conclusion

Barley is an economically important crop plant, the fourth cereal worldwide in terms of the planting area, utilized almost 60% as animal feed, around 30% for malt production, 7% for seed production and only 3% for human food [11, 12]. In recent years, the malt derived from the germinated barley is the key material for the malting represents the most economically favourable application for beer brewing [13]. There is tremendous genetic research on barley at morphological, biochemical and molecular level for development of superior barley varieties. However, detailed analyses should be performed to investigate for the environmental extrapolation of laboratory developed lines. The relationship between environmental effects and genetic studies, especially field studies will provide knowledge about interaction of environment and genetically developed varieties. We tried to cite as many papers as possible. Yet we apologize to authors whose works are gone unmentioned in this chapter.

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