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REVIEW ON *BARLEY* (*HORDEUM VULGARE* L.) IMPROVEMENT FOR DROUGHT STRESS TOLERANCE IN ETHIOPIA

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Abstract

Barley is very important food crop in the highlands of Ethiopia. Barley grain is important source of malt and food for human. In Ethiopia traditionally barley used for making of local food (injera), local bread (dabo), roasted grain(kolo), boiled grain (nifro), porridge(genfo) and other types of local beverages (beer). The byproducts are useful for livestock and poultry feed. Barely production significantly threatened by both biotic and abiotic stress. This study targeted to review on the abiotic factors of barley crop drought tolerance mechanisms, molecular bases of crop response and barely breeding strategies in moisture limited environment. Response and drought coping mechanisms in barley; re-establishment of cellular homeostasis, reduced stomatal density and correspondingly low stomatal conductance a higher level of abscisic acid, Dehydrins and osmotic adjustments. There are drought-associated phenotypic traits and their interrelationships in barley, might be positive or negative. Some of the traits which are reliable indicators of drought tolerance; early flowering, root length, root density, root proliferation, root-shoot ratio and root penetration, root volume and root-shoot ratio etc. measurement of quantitative data of such root traits under field conditions is difficult, their indirect evaluation via related and easily measured traits can be helpful. Root traits, such as root length, root volume, and root dry weight; and shoot traits, such as plant height and shoot dry weight, are closely related mainly under low-moisture stress. The root traits are inversely related to plant height and shoot dry weight. Under moisture-stress conditions, plants allocate extra photosynthetic resources to roots. Grain number per spike, grain weight per spike, grain number per plant, grain weight per plant, and thousand-grain weight are pertinent traits often associated with low-moisture stress in barley. Nevertheless, a cultivar may be developed with multiple desirable traits by combining target alleles (QTL) obtained from various gene pools via introgression. However, it is less effective for traits associated with drought tolerance, which is influenced by multiple QTL and genetic interactions.

Keywords: Barley crop, stress tolerant, barley production, abiotic factors, moisture stress.

Introduction

Barley (*Hordeum vulgare L.)* is a self-pollinating diploid species (2n = 14) and is a descendant of wild barley (*Hordeum spontaneum C Koch*). It ranks fourth among cereals after wheat*,* maize*,* and rice. Barley is believed to be the oldest domesticated crop long before other cereals were known in recorded history (Bekele *et al.*, 2005). According to the report of FAOSTAT (2020), in 2019/20 crop year, global barley production was approximately 156.4 million metric tons. In the 2019/2020 growing season, 950,742.01 hectares of land was covered by barley in peasants' farms in Ethiopia with a total harvest of 2, 378, 0102.92 tons (CSA, 2020). Its productivity (2.66

t/ha) is lower compared to that of other barley-producing countries such as United Arab Emirates, Belgium and Netherlands (8, 7.59 and 7.0 t/ha, respectively) (FAOSTAT, 2018). In Ethiopia Barley is cultivated from 1400 to over 4000 m.a.s.l (Asfaw, 2000). Even though barley is cultivated in almost all parts of the country, Arsi, Bale, Shewa, Gojam, Gonder, Welo, and Tigray are the most important barley producing regions accounting for more than 85% of the country's total production (Abebe *et al*., 2010).

Demand and production of barley are growing for various reasons: genetic diversity, wide adaptability, model crop for molecular research and a wide range of uses, such as food, beer, and feed. It is used as a food, feed and beverage in more than 20 different ways in the country. Drought stress defined as a shortage of water which induces dramatic morphological, biochemical, physiological and molecular changes, which reduce plant growth and crop production. The existence of genetic diversity has special significance for improving productivity and maintenance of diversity in a country like Ethiopia (Worede *et al.,* 2000). As stated by Kebede *et al.,* (2019), barley productivity and production can be further enhanced by exploiting available genetic diversity to develop improved cultivars for diverse environments. Therefore, the objective of this study is to review an apt literature on drought tolerance mechanisms molecular bases of crop response and barely breeding strategies in drought (moisture limited environment).

Discussion

Effect of Drought Stress on Barley

Barley adapts to a severe water regime compared with other cereals(Samarah, 2005). Prolonged mild or severe drought stress resulted in major losses in grain yield of barley where rain is scarce, irregularly distributed and variable from one year to another. However, its productivity is limited by terminal drought stress during grain filling. Barley is more sensitive during and just before spike emergence and during anthesis and the initial stages of grain development. The effect of drought stress from the beginning of grain filling to maturity may be detrimental to grain development (grain abortion) and yield. Post-anthesis drought stress may decrease the fertility of late-formed tillers and whether these late-formed tillers contribute to grain number and yield of barley. Drought stress decreases grain-filling duration and decreases grain yield (fig 1).

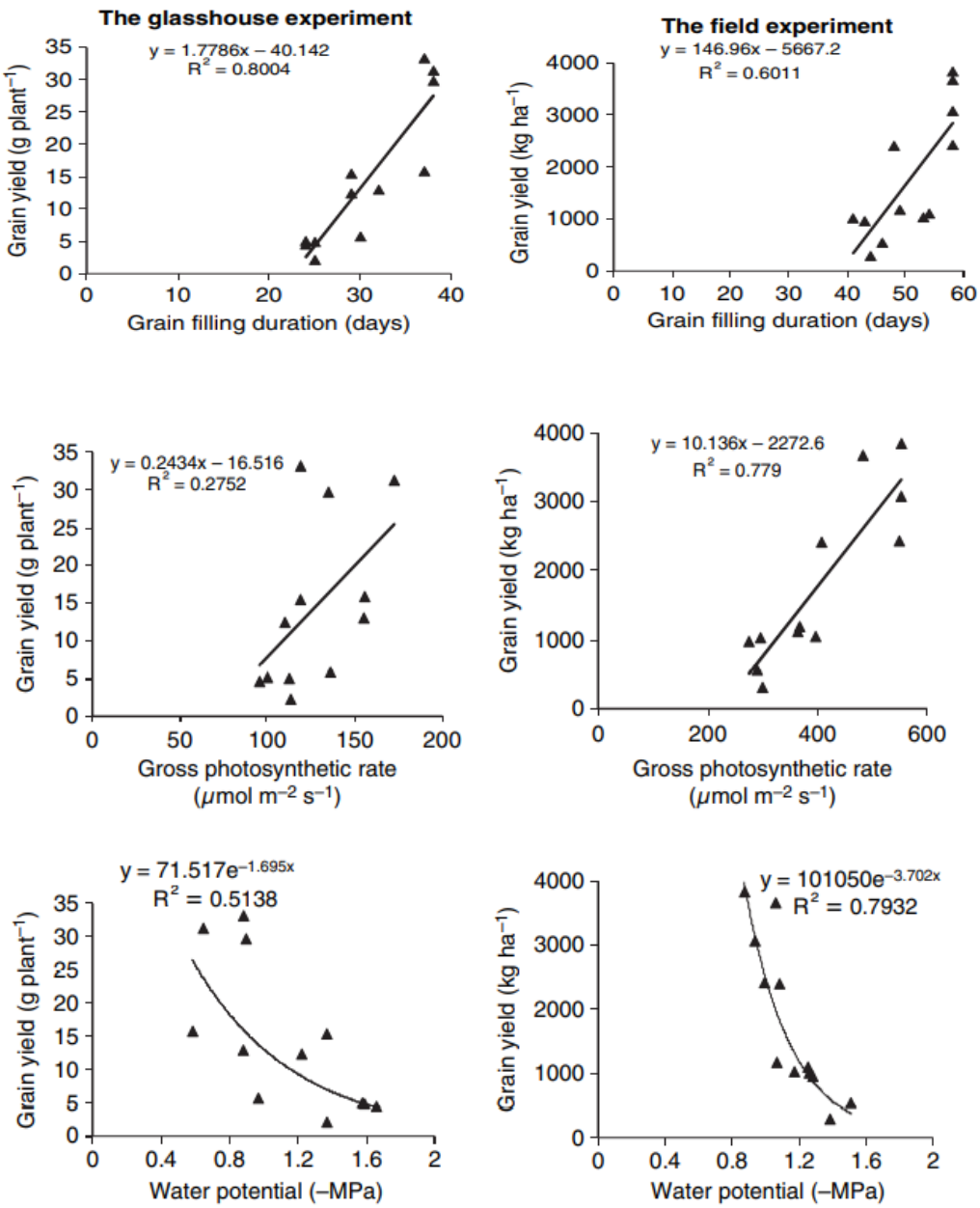

Fig 1. Relationship between grain yield and grain filling duration (a, b), gross photosynthetic rate (c, d) and water potential (e, f) of barley cultivars exposed to three watering treatments in the glasshouse and field experiment, respectively, at Jordan University of Science and Technology during the 2005–2006 growing season(adapted from: (Samarah *et al.*, 2009).

Drought and high temperatures increase photo respiratory losses, there by impacting photosynthesis, causing crop yield losses and food insecurity (Mir *et al.*,

2012; Mittler, 2006; Qin *et al*., 2011). Nutrient uptake, translocation and metabolism are reduced because of the adverse effects of drought on transpiration, size of the source and sink tissues, phloem loading, assimilate translocation, dry matter partitioning, pollination and seed set, though the level of effects differs with plant species, growth stage, and the intensity of drought (Farooq *et al*.,2009). Starch biosynthesis of simple carbohydrates occurs in cereals with the aid of sucrose synthase, adenosine diphosphate-glucose-pyro-phosphorylase, starch synthase, and starch branching enzyme (Taiz and Zeiger, 2006). Water deficit and salinity, especially under high light intensity or in combination with other stresses, disrupt photosynthesis and increase photorespiration, altering the normal homeostasis of cells and causing an increased production of reactive oxygen species (ROS) (Miller *et al*., 2010). The ROS are said to play a dual role in the response of plants to abiotic stresses, functioning as toxic by-products of stress metabolism and important signal transduction molecules (Miller *et al*., 2010).

Mechanisms of drought resistance in barley

Plant morphological, physiological, and biochemical changes are involved in response to low-moisture stress (Anjum *et al*., 2011; Shabala and Pottosin, 2014, and Kebede *et al.,* 2019). Adaptation strategies in crops against drought stress are revealed via interaction between phenology and pattern of water use (Sekhon *et al.,* 2010; Wahid *et al.,* 2007). These include improved water- uptake, water-use efficiency (WUE) and harvest index; reduced photosynthetic and respiratory activities (Zhang *et al.,* 2005b); decreased number of tillers (El Soda *et al.,* 2010); and hastening of maturity (Singh et al., 2010). Guo *et al.,* (2009) conjectured that drought tolerant barley genotypes might have gained their drought tolerance under drought stress through the re-establishment of cellular homeostasis, the enhancement of functional and structural protection of proteins and membranes and the adjustment of stomata. Drought tolerant genotypes exhibit improved expression of enzymes associated with the reallocation of the metabolites from older to younger leaves compared to drought sensitive genotypes (Forster et al., 1990). Stomatal closure under low-moisture stress limits CO2 diffusion to the chloroplasts, reducing both internal CO2 concentration (Chaves *et al.,* 2003) and enzymatic activity. Caine *et al., (*2018) have developed a high-yielding rice cultivar (IR64) with fewer stomata by manipulating the level of a developmental signal. They overexpressed the rice epidermal patterning factor OsEPF1 and created plants with substantially reduced stomatal density and correspondingly low stomatal conductance. The low stomatal density rice was more able to conserve water (about 60% of the normal amount of water used between weeks 4 and 5 post-germination) (Caine *et al.,* 2018).

The low stomatal density rice produced equivalent or even improved yields, despite a reduced rate of photosynthesis under some conditions (Caine *et al.,* 2018).

Under drought stress, a higher level of abscisic acid (ABA) is required for activation of enzymes vital for translocation of accumulated water-soluble carbohydrates than under a non-drought-stress environment in cereals, such as wheat and barley, which alters the harvest index (Maiti and Satya, 2014). Presence of the transcript machinery of biosynthesis and de-conjugation of ABA in developing endosperm and embryo of barley grain under low-moisture stress increase ABA and starch levels. The ABA and starch levels are positively correlated in shortened seed-filling period (Seiler *et al.,* 2011). Dehydrins (Dhn), a distinct biochemical group of late embryogenesis-abundant proteins, characterized by the presence of a lysine-rich amino acid motif (the K-segment), are immunologically distinct plant proteins induced during drought and other environmentally imposed dehydration (Kosová *et al.,* 2007). Thus, these proteins are suitable markers for the reduction of water activities in plant tissues (Tommasini *et al.,* 2008). Normally, dehydration-shock treatments are more reliable, faster and easier approaches than drought-induced dehydration for detecting and characterizing alleles with a variable response under drought conditions, as reported in Arabidopsis (Oono *et al.,*2003) and barley (*Talamè et al.,* 2007). Osmotic adjustments occur because of the accumulation of solutes, such as amino acids (proline) and sugar alcohols (mannitol) in the cytoplasm, which decrease the osmotic potential of the cytosol and sustain cell-water content by increasing water uptake. Hence, leaves capable of high osmotic adjustments maintain more water compared with leaves with low osmotic adjustments (Blum, 2011a). Salicylic acid, auxins, gibberellins, cytokines and ABA aid plant growth and regulate plant responses to drought. Polyamines, citrulline and other enzymes also act as antioxidants to lessen drought effects (Farooq *et al.,* 2009).

Genotype to phenotype breeding methods for drought tolerance in barley Genotype-by-environment interaction plays an important part in choosing an appropriate breeding strategy. Our understanding of how genotype controls phenotype is limited by the scale at which we can precisely alter the genome and assess the phenotypic consequences of each perturbation (Roy *et al.,* 2018). Breeding for drought tolerance can be conducted under optimal or less than optimal growing conditions. As stated by Baum *et al* (2007), two contrasting philosophies have been used to breed crops for drought tolerance. The first uses selection under optimum growing conditions, and is based on the assumption that an increased yield potential will have a carryover effect when the improved cultivars are grown under less favorable conditions. The second uses direct selection in the presence of drought within the target environment, and can take two forms: (i) selection for physiological or developmental traits (analytical breeding), and (ii) direct selection for grain yield (empirical or pragmatic breeding). The first philosophy has failed to produce convincing results. In barley, scientists consistently found a negative relationship between yield potential and yield under stress conditions (Van Oosterom *et al*., 1993) reported that total seasonal rainfall was most strongly correlated with the grain yield of Harmal (cold tolerant), accounting for 62.8% of the variance and that for Arabi Aswad (cold sensitive), rainfall from

November to January gave the best fit, accounting for 61.8% of the variance. They further indicated that December and January rainfall made the highest contribution to the yield of both cultivars; the contribution of March rainfall tended to be negative. If a breeding program is conducted under low-moisture stress, as it will not produce cultivars that can do well under optimal conditions. However, suggestions have been made to improve the reliability of breeding under such unfavorable environments by more precise estimation of experimental error (Singh *et al*., 2003) and by relying on the magnitude of heritability of a trait. Application of a realistic genotype-to-phenotype model (Cooper *et al*., 2005, 2009) is helpful in improving genotype selection.

Use of molecular markers and QTL in barley breeding

Marker assisted backcrossing (MAB), marker-assisted recurrent selection (MAS) and genome-wide selection (GWS) are among the useful approaches for developing drought-tolerant cultivars. Major QTL can be used for introgression genes controlling a trait of interest into the target genotype via MAB without the noise of deleterious genes (Gupta *et al*., 2010). Marker-assisted recurrent selection is appropriate for an F2 base population of self-pollinated crops, such as barley (Bernardo, 2008) and advantageous over other approaches when a trait is governed by more than one QTL. This procedure allows inter-mating in subsequent selection stages to increase the frequency of desirable alleles (Eathington *et al*., 2007; Ribaut and Ragot, 2007). GWS uses genome-wide markers for genotyping a phenotyped population. Genotypes are selected in a subsequent recurrent selection cycle on the basis of "genomic-estimated breeding value" (GEBV) (Meuwissen *et al*., 2001). The GWS can be used in both self- and cross-pollinated crops with some modifications (Bernardo, 2008); it reduces the frequency of phenotyping (Rutkoski *et al*., 2010) and allows the development of lines possessing novel multi-genes controlling drought tolerance and others desirable traits. Association of drought-related QTL with molecular markers is important for successfully carrying out MAS. Molecular markers, such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR), diversity array technology (DArT) and single nucleotide polymorphism (SNP), are useful for mapping QTL for drought-tolerance-associated traits in barley (Akash, 2014; Forster *et al*., 2004; Wenzl *et al.,* 2004). Fine-focus mapping can be used for drought-tolerance-related genomic regions and the location of the gene underlying a QTL of interest can be narrowed down. During the past, different molecular markers have been used to study genetic basis of drought tolerance in barley; for instance, QTL have been identified for metabolites using SNP markers (Li *et al*., 2013; Templer *et al*., 2017), shoot and root traits using SSRs (Arifuzzaman *et al*., 2014), chlorophyll content, initial fluorescence (Fo), maximum fluorescence (Fm), variable fluorescence (Fv) and maximum quantum efficiency of PSII (Fv /Fm) using SSRs and AFLPs (Guo *et al*., 2008).

RNA Interference technologies breeding methods for drought tolerance in barley

The RNAi is an important mechanism of silencing specific target genes. Basically, in this process, cells use short, double-stranded RNAs (ds RNAs) to recognize specific messenger RNAs (mRNAs) and destroy/degrade them enzymatically, thus keeping them from being translated into a protein (González-Alegre, 2010), hence the term "gene silencing." Two types of small RNA molecules (microRNA (miRNA) and small interfering RNA (siRNA)) are central to RNA interference. Using RNAi, the traits that have been improved in barley are barley yellow dwarf virus (BYDV) by targeting the BYDV PAV gene (Wang *et al*., 2000) and amylose by targeting the SBE IIa and SBE IIb genes (Regina *et al*., 2010). Zalewski *et al*. (2010) used RNAi-based silencing of HvCKX1 gene which decreased the CKX level, especially in those organs that showed the highest expression, i.e., developing kernels and roots, leading to higher plant productivity and higher root mass. The same type of RNAi construct was applied to silence HvCKX2 gene and analyze its function (Zalewski *et al*., 2012).

Drought stress-related genes have not been targeted in barley and it opens great opportunity for barley researchers to work in this direction. The RNAi technique has been used in the context of drought stress tolerance in other crops. For example, Wang *et al*. (2011) identified drought-responsive micro-RNAs (miRNAs) in *Medicago truncatula* and reported that 22 members of 4 miRNA families were up-regulated and 10 members of 6 miRNA families were down-regulated in response to drought stress. Sunkar and Zhu (2004) described the role of miRNAs in response to abiotic stresses in Arabidopsis, whose seedlings were exposed to different abiotic stresses, such as drought, cold, salinity, and oxidative stress. They found that miR393 was strongly up-regulated by cold, dehydration, high salinity and abscisic acid (ABA) treatments. Hwang *et al*. (2011) identified drought stress-responsive miR171 family members (named miR171a, miR171b and miR171c) potato. Trindade *et al*. (2010) identified several conserved miRNAs having differential expression in *M. truncatula* plants, as miR169 was down-regulated in roots, whereas miR398a/b and miR408 were strongly up-regulated in both shoots and roots under water-deficit conditions. In canola (*Brassica napus* L.), the RNAi-suppression of farnesyl transferase genes FTA or FTB decreased stomatal conductance and thereby transpiration, which resulted in higher yields (Wang *et al*., 2005, 2009). Jian *et al*. (2010) identified stress-related miRNAs in rice (*O. sativa L. ssp.* Japonica CV 9522) seedlings that were exposed to cold, dehydration, salinity and abscisic acid stresses. Zhao *et al*. (2007) discovered several stress-related miRNAs in rice and only two miR393 and miR169g were found to be related to abiotic stress, both of which were up-regulated by dehydration.

Genome editing technologies breeding methods for drought tolerance in barley

Targeted genome editing using artificial nucleases has the potential to accelerate basic research as well as plant breeding by providing the means to modify genomes rapidly in a precise and predictable manner (Bortesi and Fischer, 2015). In 2012, a new genome-editing system emerged, which is different from the recombinant DNA technology in that no transgenes are used. The new system is called "clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9" (CRISPR/Cas9). The system has generated much interest among biologists. In fact, in 2015, Science magazine labeled it "Breakthrough of the Year." The new designer nuclease-mediated genome-editing technology allows modification of genetic makeup of crops much faster and more precisely than the transgenic technology. The CRISPR, a type of adaptive immunity developed via evolution in prokaryotes (Mojica *et al*., 2000), is a site-specific gene-editing tool, which induces mutations in plants (Bolotin *et al*., 2005; Mojica *et al*., 2005). According to Gao (2018), conventional plant breeding will not be able meet increasing food demands and other environmental challenges. GAO (2018) further stated that CRISPR technology was erasing barriers to genome editing and could revolutionize plant breeding and that fully benefit from the CRISPR revolution, focus should be on resolving its technical and regulatory uncertainties. Gene editing has been successfully employed to develop potatoes that are low in acrylamide a potential carcinogen (Clasen *et al*., 2016). Recently, scientists have developed a transgene-free powdery mildew resistant variety of tomato ("Tomelo" by use of genome editing tools (Nekrasov *et al*., 2017). In barley also, the genome-editing tool, CRISPR/Cas9, which is efficient, simple, and low-cost and involves DNA and RNA, has been used to edit gene(s) of interest and alter metabolic pathways, with 10–23% mutation rate (Lawrenson *et al*., 2015; Ma *et al*., 2015). With its acceptance and wide range of application, CRISPR/Cas9 system is susceptible to off-target effects (e.g., side effects on nearby genes) that may result in undesired mutations (Song *et al*., 2016; Zhu *et al*., 2017). For instance, CRISPR-Cpf1 system, which is more efficient in avoiding off-target effects unlike CRISPR-Cas9; Cpf1 is a smaller and simpler endonuclease than Cas9 (Hu *et al*., 2017; Zaidi et al., 2017). Application and efficiency of CRISPR-Cpf1 and other emerging genome-editing technologies/tools to specific traits of interest (e.g., drought tolerance) are indispensable to barley improvement programs. Currently, genome-editing tools, e.g., CRISPR-Cpf1, have not been applied in barley. Barley researchers would need to catch up with other cereal crops as far as the use of CRISPR-Cpf1 is concerned.

Scientists are continuing to innovate in the area of genome editing. Quite recently, Roy *et al*. (2018) described a CRISPR/Cas9-based method for "multiplexed accurate genome editing with short, trackable, integrated cellular barcodes" (MAGESTIC) in yeast. This method is supposed to be an improvement over the

CRISPR/Cas9 system. However, additional research is needed to test its amenability to crop plants. Scientists working with or planning to work with genome-editing systems should be cautioned that very recently (July 25, 2018), the Court of Justice of European Union has ruled that gene-edited crops were subject to the same stringent regulations as conventional genetically modified (GM) organisms (Callaway, 2018).This decision was made despite the fact that scientists contended that gene-editing techniques (e.g. CRISPR-Cas9) should be regarded as mutagenesis like irradiation and that their products should be exempt from the 2001 directive, as they do not involve foreign DNA (transgenic material). This ruling might dampen scientists' enthusiasm regarding gene-editing techniques in European Union. On the contrary, US Department of Agriculture has cleared a common white button mushroom (*Agaricus bisporus*) that has been genetically modified with the CRISPR-Cas9 system to resist browning (Waltz, 2016). This gene edited mushroom was created by targeting the family of genes that encodes polyphenol oxidase enzyme that causes browning.

Conclusion and Future Directions

Cultivars development with drought tolerance is a difficult task, as complex plant genetic and metabolic pathways are involved in plants' response to low-moisture stress, including differential expression of alternative alleles of major genes in a QTL under stress and non-stress conditions. Use of parents with contrasting phenotypic traits and mutants with differential responses to low-moisture stress is essential for improved resolution of QTL mapping and identification of genes for drought-associated traits. A wide genetic base of RILs, stable (non-segregating) population, and focus on targeted environment are the necessary conditions to identify and effectively use QTL in MAS for drought tolerance. Alleles with a variable expression under low-moisture environments can be identified more successfully using dehydration-shock treatments than drought-induced dehydration. Physiological, morphological, and agronomic traits can be used for drought-stress studies in barley. Normally, physiological traits are governed more by drought-responsive QTL compared with agronomic traits. Thus, knowledge about plant constituents associated with low-moisture stress is helpful in breeding for drought tolerance.

Early vigor, plant height, SWX, grain yield, thousand-grain weight, root volume, root length, shoot dry weight, wilting score, ABA concentration, water-soluble carbohydrate concentration, and chlorophyll content are important traits associated with low-moisture stress. Among these traits, early vigor, plant height, SWX, grain yield, and thousand-grain weight are more pertinent than other traits; these are considered consensus traits in barley breeding for drought tolerance. Efficient use of genetic resources and improving efficiency of breeding for drought tolerance would serve barley breeders well in developing drought-tolerant barley cultivars to combat climate change. To combat climate change, barley breeders could employ techniques

similar to those used by Caine et al. (2018) to develop low stomata density barley; plants with fewer stomata are drought tolerant and more water-use efficient.

Genome-editing systems, such as CRISPR/Cas9, CRISPR-Cpf1, and MAGESTIC, once perfected, offer opportunities to appropriately edit barley genome to streamline metabolic pathways involved in drought tolerance. Application of such modern techniques is expected to have significant impact on barley improvement programs. Barley breeders as well as breeders of all other cereals would need to continue to develop and use innovative technologies to be able to meet the production challenges that lie ahead.

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