



Genetic Improvement of Wheat and Barley Using Transgenic Approaches **23**

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Abstract

Wheat and barley are contemplated to be the most imperative cereal crops around the world considering their nutritional values. From recently available huge amounts of databases on structural and functional genomics of wheat and barley, biologists can concentrate on modifying structure and functions of certain primary genes with the use of genetic engineering methods. Genetic modification enables the incorporation and expression in the cells of living organisms of distinct genes of interest, bypassing, if necessary, the obstacles of sexual incompatibility. The target traits for genetic transformation are usually linked to the production of adequate food for ever-increasing global human population; improvement of plant architecture; providing tolerance to bacterial, viral, and fungal diseases; and the production of varieties that could thrive in extreme environmental conditions such as high temperature, salinity, drought, and heavy metal stress. Through this chapter, we have tried to summarize how the target traits could be improved by genetic engineering using transgenic as well as recent genome editing technologies and will provide a review of current and future applications in wheat and barley research.

Keywords

Wheat · Barley · Genome editing · Transgenics · Abiotic stress · Biotic stress · Quality

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P. L. Kashyap et al. (eds.), *New Horizons in Wheat and Barley Research*, https://doi.org/10.1007/978-981-16-4449-8_23

623

23.1 Introduction

Poaceae family members comprise crops of vast agricultural and economic value, like wheat, corn, rice, and barley. Wheat (*Triticum aestivum* L.) is widely used in the production of human food (74%), 16% is used as animal feed, and the remaining portion is used in agricultural applications. To satisfy the food requirements of an ever-expanding human population, global wheat demand needs to be doubled over the next few decades (Hall and Richards 2013). Barley is the fourth largest cereals in terms of production area and makes a huge impact worldwide, with production volumes reaching almost 130 million metric tons each year (<http://faostat.fao.org>). Barley genome size is large and highly repetitive (~5.1 Gb) distributed over seven chromosomes. Barley proves to be a model species for genetic transformation studies because of its characteristic features like it is diploid in nature and its genome is less complex than other cereal species, availability of large germplasm resources and recent developments in enhanced genetic transformation efficacy. In addition to its use in human consumption, barley is used for brewing and distillation industry and commonly as animal feed supplement. Barley grains have also been found to be accepted as a bioreactor in medicinal protein formulations. Latest advances in the genomics of barley and wheat during the last decade focused on the accumulation of a vast number of EST data sequences; mapping of molecular markers linked to yield and quality traits; BAC libraries; availability of latest high-throughput platforms for transcriptomic, proteomic, and metabolomic analysis; and availability of knockouts and mutant repositories. Transgenic technology assists in the functional validation of genes and expression networks linked to agronomic traits.

23.2 Genetic Transformation in Wheat and Barley

In order to address the rising demand for food security worldwide, there is a clear need for standardization of effective transformation methods for targeting the desired DNA into cereal genome. Transgenic technology in plants includes the editing of the plant genome by different methods which result in incorporation, expression, and transfer of the inserted gene to the next generation. These transgenics provide resistance to abiotic and biotic stresses; improved quantity and quality of grains also serve as a bioreactor or biofactory for producing pharmaceuticals and chemical compounds for industrial application. The delivery of DNA to plants can be accomplished through an indirect transformation approach using *Agrobacterium tumefaciens*-mediated transformation method. Under natural conditions, cereals have been observed to be recalcitrant to *Agrobacterium* infection. However, during the last two decades, tremendous efforts lead to workable and reproducible protocols for DNA delivery into barley and wheat by *Agrobacterium*-mediated transformation method. Another popular method is by particle bombardment also known as biolistic transformation, introduced in the 1980s. This approach was found ideal for genetic transformation of monocotyledons which otherwise were once found to be recalcitrant for *Agrobacterium* infection (Klein et al. 1987). Despite differing ploidy levels,

wheat and barley have seven primary basic chromosomes in each genome and share comprehensive conservation among homologous chromosomes (Mayer et al. 2011). Genetic transformation is currently feasible in wheat and barley; however, wheat is considered difficult to transformation than barley (Harwood 2012). The first documented *Agrobacterium*-mediated wheat transformation was to be followed in 1997 (Cheng et al. 1997). However, even after this first encouraging study, the transformation of wheat by *Agrobacterium* proved to be difficult and inefficient (Harwood 2012). Risacher et al. (2009) reported an effective in planta transformation of wheat with the *Agrobacterium*-mediated inoculation process. However, this technique demanded expertise and was not generally accepted. Ishida et al. (2015) described and reported an efficiency of 40–90% using immature embryo as explants. There is another powerful genetic transformation method in wheat, that is patented and available via a license from Japan Tobacco Inc. (<http://www.jti.co.jp>), as two systems, the PureIntro™ and the more complex PureUpgrade™. In barley, different tissues were used as source of explants for biolistic transformation like immature embryos (Kartha et al. 1989), microspore-derived embryos (Jhne et al. 1994; Carlson et al. 2001), endosperm, meristematic cells from leaves (Shirasu et al. 1999), and shoots (Zhang et al. 1999).

23.3 Transgenic Wheat and Barley for Tolerance to Abiotic Stress

Barley is among the oldest growing crops worldwide with considerable potential for adaptation. That demonstrates exceptional tolerance to salinity, drought, and fungal infections, which makes barley a model organism for study of stress biology. Barley has natural stress tolerance, which contributes to an increasing interest in the exploration of stress-responsive genes by genomics and other omics studies. The majority of plant proteins responsible for stress tolerance are transcription factors and gene coding for antioxidant enzymes, osmolytes and transporters. These transcription factors have been identified and cloned in barley and have been shown to be functionally useful in stress tolerance for the generation of transgenic lines (Table 23.1). Overexpression of barley TFs like *HvCBF4* (Oh et al. 2007), *HvDREB1*, and *HvWRKY38* in different transgenic lines results in enhanced resistance to salinity and drought as these proteins result in enhanced expression of stress-related gene and improved DNA binding affinity. LEA proteins are known for their hydrophilic nature, large size, and fast aggregation in the desiccation period of seeds during abiotic stress response (Bhatnagar-Mathur et al. 2008). HVA1 is a LEA protein which shows its expression in aleurone layer and plays critical role for enhancing abiotic stress tolerance in plants. In transgenic spring wheat, HVA1 enhanced drought tolerance, increased biomass production, and efficiently improved water usage during drought stress conditions (Sivamani et al. 2000). In wheat, overexpression of the *TaNAC69* gene (Xue et al. 2011) resulted in drought tolerance; its expression was regulated by two promoters of the barley dehydrin gene, *HvDHN8s* a constitutive promoter and *HvDHN4s* that is inducible under drought

Table 23.1 Transgenics developed for abiotic stress tolerance

Gene	Function	Trait improved	References
<i>TaERF3</i>	ERF transcription factor	Tolerance against drought and salt	Rong et al. (2014)
<i>AISAP</i>	Stress-associated protein	Higher tolerance to dehydration and salt	Ben Saad et al. (2011)
<i>P5CS</i>	D1-pyrroline-5-carboxylate synthase	Salt tolerance	Sawahel and Hassan (2002)
<i>Mtd</i>	Mannitol biosynthesis	Enhanced salinity tolerance	Abebe et al. (2003)
<i>AtNHX1</i>	Vacuolar antiporter gene	Improved growth under high salinity	Moghaieb et al. (2014)
<i>HVA1</i>	The ABA-responsive gene	Improved water use efficiency	Sivamani et al. (2000)
<i>HKT1</i>	High affinity potassium transporter	Enhanced growth at higher NaCl (200 mM) conditions	Laurie et al. (2002)

conditions. These plants exhibited increased buildup of biomass under water-deficit condition along with improved water usage efficiency during initial stages (Xue et al. 2011). Overexpression of rice gene *OsMYB4* in barley results in transgenic lines showing increased frost tolerance and enhanced germination rate with less effect on plant growth during low temperature conditions (Soltesz et al. 2012). The overexpression of *AtCIPK16* in barley showed improved salinity tolerance, as well as an increase in biomass after long-term exposure high salt stress for 30 days (Roy et al. 2013). Their findings suggest that *AtCIPK16*-induced salt tolerance is accomplished by regulation of the transcription factor and signaling of phytohormones. Two members of the homeodomain zipper group from wheat (*TaHDZip1-2* and *TaHDZip1-5*) were delivered in wheat and barley as transgenes. Transgenic lines were obtained with improved drought and frost tolerance (Gonzalez et al. 2019). Aluminum (Al^{3+}) is harmful to plants in acidic soils. *ALMT1* gene overexpression in barley plants contributes to vigorous root development in transgenic lines, when such plants were grown in a polluted hydroponic culture with high aluminum level. However growth of control plants was reserved along with visible deformities in root apices exposed with metal stress conditions (Delhaize et al. 2004). Fujii et al. (2012) explained that insertion of 1 kb upstream of coding region changed expression patterns of *HvAACT1* and leads to improvement in Al^{3+} tolerance in sensitive barley cultivar by using *Agrobacterium*-mediated transformation process. Expression of transgenic bet A gene in wheat yielded higher GB accumulation levels and substantial protection of plants, during salt stress. Some of these transgenic lines showed higher glycine betaine level, lower solute potential and Na^+/K^+ ratios, and less damage to cell membrane (He et al. 2010). DREB/CBF overexpression resulted in protective effect on integrity of cell membranes (Morran et al. 2011). Overexpression of *DREB2* and *DREB3* genes using constitutive duplicated promoter *CaMV35S*, also drought-inducible promoter, and maize *ZmRAB17* promoter resulted in enhanced tolerance for water deficiency and frost tolerance. The

expression of DREB genes powered by the promoter *ZmRAB17* was more resistant to drought stress without adverse plant growth and developmental consequences. The overexpression of *GmDREB1* in transgenic wheat lines resulted in improvement of traits related to yield along with improved salinity tolerance (Jiang et al. 2014). These findings indicate that *GmDREB1* controls the expression of proteins related to osmotic and oxidative stress, which reduce the incidence of cell damage due to high salinity. Two DREB/CBF genes, *TaDREB3* and *TaCBF5L*, were transformed into barley and wheat using the stress-inducing promoters HDZI-3 and HDZI-4. Inducible expression of these promoters in leaves of transgenic wheat and barley lines was tested during drought and cold stress conditions (Yang et al. 2020). During drought stress and freezing conditions, expression of downstream *TaCBF5L* gene was upregulated in transgenic wheat seedlings. The application of HDZI-4 promoter-driven *TaCBF5L* in wheat results in improvement in yield during drought stress. Transgenic barley overexpressing subfamily HKT transporter (*HvHKT2;1*) shows increased development of biomass when exposed to salt stress possibly due to Na⁺ exclusion or excessive Na⁺ accumulation in leaves of plants (Mian et al. 2011). Expression pattern analysis showed that polyethylene glycol (PEG), H₂O₂, and Fe-ethylenediamine di(*o*-hydroxyphenylacetic) acid induced the expression of *TaFER-5B* in wheat. WRKY transcription factors tend to enhance stress tolerance. *AtWRKY30* was cloned and expressed in wheat (El-Esawi et al. 2019), and results showed that *AtWRKY30* promotes resistance to drought and heat in transgenic lines by inducing antioxidant properties, synthesis of osmolytes, and expression of genes related to stress response. *AtWRKY30* may act as a possible candidate for improvement of stress tolerance in wheat.

23.4 Biotic Stress Tolerance in Transgenic Wheat and Barley

Biotic stress is the disruption of plant system by living organisms, including fungi, protists, bacteria, insects, and viruses. Pathogens are accused of a substantial decline in global food supply and a major obstacle to resistant seed breeding. A variety of biochemical, genetic, and molecular processes are considered to include plant resistance mechanisms to different pathogens and insect pests. The defensive mechanism has been identified as innate and systemic reaction of plants. The defense system of plants includes external barriers like cell walls and epidermis, and chemical defense involves compounds like metabolites, phenolics, nitrogen compounds, proteins, and enzymes. Insect infestation is a major factor for loss of quantity and quality of wheat grain. The *Sitophilus granarius* wheat weevil is a major insect pest of the crop and is responsible for substantial yield loss. A synthetic avidin gene (*Triticum aestivum* L.) cv was transformed into spring wheat by using biolistic bombardment method (Table 23.2). Avidin protein accumulation was observed in transgenic plants with high levels of expression in seeds (Abouseadaa et al. 2015). An insect bioassay has verified the functional integrity of avidin. The barley gene *HvNAC6* acts as a regulator against the *Blumeria graminis* f. *hordei* pathogen in barley. Transgenic approach to silence the expression *HvNAC6* with

Table 23.2 Transgenics developed for biotic stress tolerance in wheat and barley

Name of gene	Function	Trait improved	References
<i>TaPIEPI</i>	Ethylene responsive factor	Resistance to <i>Bipolaris sorokiniana</i>	Dong et al. (2010)
<i>Nla</i>	Nuclear inclusion protein	Hairpin RNA confers immunity to infestation caused by wheat streak mosaic virus	Fahim et al. (2010)
<i>afp</i>	Antifungal protein	Enhanced fungal (<i>Erysiphe graminis</i>) resistance	Oldach et al. (2001)
<i>RCH8</i>	Chitinase	Leaf extract of transgenic lines shows resistance to wheat scab	Wu et al. (2001)
<i>Pm3b</i>	Powdery mildew resistance	Improved resistance against powdery mildew	Kalinina et al. (2011)
<i>TaPERO</i>	Peroxidase	Increased powdery mildew resistance	Altpeter et al. (2005)
<i>pin2</i>	Serine proteinase inhibitor	Nematode resistance	Vishnudasana et al. (2005)

help of using RNA interference (RNAi) technology helps plant biologists to understand the function and role of *HvNAC6* in barley plants (Chen et al. 2013). Overexpression of the same gene results in improvement in barley resistance to the *Ramularia* leaf spot (McGrann et al. 2015). Chitinase gene expression increases resistance of plant species to fungal diseases. Constitutive expression of class II barley chitinase enhances resistance to *Erysiphe graminis* in wheat (Bliffeld et al. 1999) and *Fusarium graminearum* (Anand et al. 2003; Shin et al. 2008). RNA interference (RNAi) is effective genetic tool for speeding up plant biotechnology study and controlling biotic stress by controlling target gene expression. Transformation of double-stranded RNA expressing vector in wheat to target the mitogen-activated protein kinase gene (*PsFUZ7*) from *Puccinia striiformis* shows enhanced and sustainable stripe rust resistance (Zhu et al. 2017).

Transgenic lines that express siRNAs that target *PsCPK1*, a PKA catalytic subunit gene from Pst, showed durable resistance till the T₄ generations in case of wheat (Qi et al. 2018). Stable expression of hairpin RNAi which has a sequence homology with *PtMAPK1* from *P. triticina*, in susceptible wheat cultivars, shows effective silencing of the corresponding genes in infecting fungus and results in disease resistance (Panwar et al. 2018). Another target gene for the controlling grain aphids by RNAi in wheat was lipase maturation factor-like 2 (Xu et al. 2017), carboxyl-esterase gene (Xu et al. 2014), and *Hpa1* (Fu et al. 2014). When these aphids feed upon those transgenic lines, it leads to considerable reduction in their survival and reproduction rate.

23.5 Transgenic Improvement of Qualitative and Quantitative Traits in Wheat and Barley

In order to satisfy the increasing demand for food, along with the challenges presented by climate change, significant improvement is required in the yields and nutritional quality in majority of crops including wheat and barley. Plant yield is determined by the size and numbers of grains. During the last two decades, significant improvement in genetic and genomic approaches has been established with regard to genes affecting traits related to yield and nutritional quality in these two crops.

23.5.1 Yield

Grain size (GS) had always been the subject for selection and modern breeding in wheat. *TaGW2* shows negative effect on size of grains by controlling cell division within the spikelet. Hong et al. (2014) had used specific RNAi-based approach to suppress three *TaGW2* homolog results for substantial improvement in the grain weight and width of the bread wheat, which were usually distinguished by small grains. In wheat transcription factor, *TaNAC2-5A* helps to signal nitrogen and influx rate of nitrate and improves root growth. Similarly, another gene that codes for the gene (*TaGS2*) overexpressed in wheat triggers enhanced photosynthesis of the leaf, and an enhanced remobilization of nitrogen to grains results in increased spike number and yield of plants (Hu et al. 2018). In the transgenic wheat lines, advanced maize ADP-glucose pyrophosphorylase (*ZmAGPase*) improves photosynthetic concentrations and plant yield (Smidansky et al. 2007). Zhang et al. (2014) worked on generation of transgenic wheat by overexpression of genes which code for the enzymes, phosphoenolpyruvate carboxylase and pyruvate orthophosphate dikinase. The results indicated improved photosynthetic traits and yield. The maize gene coding for the transcription factor *Dof1* is responsible for increased PEPC expression in transgenic wheat. *ZmDof1* expression with the light-inducible promoter *RuBisCo* contributes to an increase in biomass of transgenic wheat. *TaNfY-A-B1* overexpression leads to a substantial rise in phosphorus and nitrogen intake and grain yield in wheat. Another study involving transcription factor overexpression shows a positive role of the *TaNf-YB4* on grain yield in wheat (Yadav et al. 2015).

23.5.2 Nutritional Traits

Grain is the harvested component of the wheat and barley plant, and its dietary and health characteristics are determined by its biochemical composition. Starch and protein have a huge effect on the consistency of products obtained from wheat flour. Many of the quality-related attributes have been tackled by transgenic technologies in the recent years. Weichert et al. (2010) worked with barley sucrose transporter gene (*HvSUT1*) transformed into wheat contributes to increase absorption of protein

and sucrose in grains but no suggestive improvement in level of starch. Downregulation of transcription factor *TaRSR1*, a Rice Starch Regulator wheat homolog (*OsRSR1*), negatively controls the gene expression of certain enzymes linked to synthesis of starch grains (Kang et al. 2013). Constitutive overexpression of *NtNR* gene that was overexpressed in wheat results in enhanced activity of foliar nitrate reductase and results in substantially improved protein content of seeds. Starch is made up of amylose and amylopectin, with varying degree of polymerization. The proportion of amylose in starch was found to have a strong correlation with the resistant starch content (Regina et al. 2006). Resistant starch is a part of dietary starch which shows resistance to hydrolysis by enzymes, and its fermentation takes place in the large intestine with the help of intestinal bacteria which are anaerobic in nature. RS is correlated with a variety of promotional impacts on human health. In wheat, amylose content is increased by downregulation of starch branching enzymes, *SBEIIa* and *SBEIIb* (Sestili et al. 2010). The vernalization gene, *TaVRN2*, was targeted by RNAi in wheat plants (Yan et al. 2004). To increase the flour content of the bread, a linear DNA construct consist of HMW-GS 1Bx14 gene was transferred into bread wheat by using particle bombardment method of genetic transformation (Liu et al. 2011). Transgenic plants in which starch branching enzymes were silenced produce amylose-only starch in the case of barley (Carciofi et al. 2012). In the thermotolerant fungal endo-1,4- β -glucanase, *fEBG* genes were transformed into barley along with α -amylase promoter. Transgenic barley lines showed production of β -glucanases in aleurone tissues, and activity of enzymes is retained even after 2 h of incubation at 65 °C (Nuutila et al. 1999). Transgenic barley lines that overexpress *Arabidopsis* zinc transporter gene *AtZIP1* have been developed to improve zinc uptake. Total zinc and iron content was twofold higher than control (Ramesh et al. 2004). Altenbach and Allen (2011) used RNAi approach for suppressing expression of ω -gliadins linked with WDEIA in wheat. Later Altenbach et al. in 2014 proved that transgenic lines which have reduced ω -gliadins showed improved dough qualities during various growth conditions (Altenbach et al. 2014). Whereas the downregulation of γ -gliadin genes was successfully accomplished in Bobwhite wheat, traits were transferred to other common wheat cultivars by traditional crossbreeding (Gil Humanes et al. 2012). In wheat and barley, Connorton et al. (2017) had overexpressed two wheat iron transporter (*TaVIT*) genes. They recorded that the insertion of one *TaVIT2* gene causes iron content to be increased about twofold in transgenic lines.

At the end of the century, selective genome engineering using endonucleases such as TALENs and ZFNs was introduced as a pioneering tool for the development of mutations in the target genome at specific locations. Nuclease-dependent mutagenesis is dependent on double-strand breaks generated at specific sites; these breaks were repaired by nonhomologous end joining (NHEJ) or homologous recombination (HR) with high fidelity. At the cleavage site, NHEJ sometimes results in deletions (InDels) or insertions, which ultimately lead to direct alteration of the genome. In wheat, CRISPR/Cas9 method was used for *TaMLO* editing, which is a powdery mildew resistance locus. *Blumeria graminis* f. is responsible for powdery mildew diseases, which cause major declines in wheat production, and the knocking out of

TaMLO contributes to resistance to disease (Shan et al. 2013). Zhang et al. (2017) used CRISPR/Cas9 technologies to produce *TaEDR1* wheat mutants by simultaneously knocking down a negative regulator of powdery mildew resistance from the three wheat homologs of *TaEDR1*. Zhang et al. (2016) used CRISPR method for generating mutants of *TaNAC2* and *TaDEP1* in wheat plants. One potential effect of modification of *TaNAC2* activity is the increase of grain size in reactions to stress conditions. Sánchez-León et al. (2018) used CRISPR/Cas9 technologies to minimize the number of alpha-gliadins in the durum and bread wheat lines, producing decreased immunoactivity for coeliac disease. These examples give insight into various new ways of emerging technologies for genome modification. The key results of the plant transgenesis model were to exploit and eventually use the knowledge for substantial improvement of crops.

23.6 Conclusions and Prospective Developments

In this chapter, wheat and barley transformation techniques had been discussed and explained how these crops had been genetically modified through gene overexpression, by obtaining loss or gain of function phenotypes, and by expression of antisense RNA, and most recently, change in structure and expression level of different genes have been achieved using engineered nucleases for genome editing, such as CRISPR/Cas9. The advantages of using barley for most of the transformation experiment are because it is tolerant to diverse range of environmental factors and it has easy amenability for genetic transformation. In fact, overexpression of several barley transcription factors proves to be effective to confer abiotic stress tolerance in majority of plant species and provides multiple stress tolerance. By productive partnerships between plant molecular geneticists and breeders, there is a greater chance of development of promising future prospects. The introduction of newer techniques and study of genetically modified plants for use in breeding can be converted into traditional breeding systems through introgression of genetic traits into the field.

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