# Chapter 14
# Genomic Region Analysis and Genome Editing for Grain Quality Improvement in Cereals

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## Contents



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## 14.1 Introduction

Cereals are the principal stable crops for global population and thus are the primary source of micronutrients. However, cereals have a low level of micronutrients, and majority of them are lost during processing. Around two billion people mainly in Africa, South Asia, and Latin America suffer from malnutrition or nutrient deficiency which is responsible for the death of around 24,000 people daily all over the globe (Majumder et al. 2019). To achieve nutrient security, fortification of food, especially cereals, is necessary owing to the primary source of energy and nutrition in human diet. The availability of biofortified staple food crops would be a sustainable approach to provide a nutritious diet to people having limited access to variable dietary resources.

The principal aim of the breeders is to improve the yield potential of the cereals. Improving grain nutritional quality of cereals to attain nutritional security seems to be a newer area for breeders. Conventional and molecular approaches have enabled the breeder to develop cereals with higher micronutrients. QTLs for various micronutrients, identified in rice, maize, wheat, barley, and pearl millet, have been introgressed to develop improved versions of these cereals (Mahender et al. 2016; Govindaraj et al. 2019; Gaikwad et al. 2020; Saini et al. 2020; Prasanna et al. 2020; Swamy et al. 2021). HarvestPlus, a program under CGIAR (Consultative Group on International Agricultural Research), is working on development of biofortified crops in low- and middle-income countries. Under this program, cereals like maize, wheat, rice, and pearl millet along with other important crops have been biofortified (Bouis and Saltzman 2017). The biofortified crops developed under this program are being planted by over 8.5 million farmers across Africa, Asia, and Latin America (https://www.harvestplus.org). With the combined efforts of HarvestPlus and the institutions working with it, over 300 varieties of biofortified crops have been released for commercial cultivation in 40 developing nations using conventional and molecular breeding. A successful biofortified cereal in addition to enhanced nutritional value must be high yielding and acceptable to stakeholders.

In some cases, germplasm lacks the genetic variability in the desired trait for biofortification. Therefore, genetic modification (GM) of crops is a possible way to overcome the problem. GM technology involves introduction of the desired trait from the novel source (Yadav et al. 2018). With the low acceptability of GM crops, the ongoing agricultural practices are struggling to meet the nutrient security of the increasing global population (Rani et al. 2021; Jangra et al. 2021a, b). GE has emerged as a potential tool to overcome the challenges associated with the current crop improvement technologies. GE-assisted breeding has been successfully employed to modify the trait of interest in various crop plants without introduction of any foreign gene. In a very short time, GE had a great impact on crop improvement with its high precision and efficient genetic modification (Chen et al. 2019). The era of GE began with the introduction of double-stranded breaks (DSBs) at specific sites using endonucleases like zinc figure nucleases (Kim et al. 1996), transcription activator-like effector nucleases (TALENs) (Christian et al. 2010), and

clustered regularly interspaced short palindromic repeats (CRISPR)-associated 9 (Cas9) (Jinek et al. 2012). Since its development, GE has been employed to improve various crop plants (Park et al. 2019). Recently, there is an exponential increase in the utilization of GE for crop improvement. In this chapter, we will be focusing on the advancements made in the field of grain quality improvement in cereals using genomic regions/QTLs and GE.

## 14.2 Genomic Regions/QTLs for Cereal Grain Quality Improvement

The primary requirement for breeding biofortified crops is to look into the available germplasm to identify genomic regions with higher micronutrient content. The wild relatives of the crop plants are a rich source of various micronutrients and therefore can be utilized in the breeding program. The quality traits are polygenic and are controlled by several genes. Therefore, improvement of these traits through conventional breeding is quite difficult (Jangra et al. 2017; Jangra et al. 2018). The advancement in molecular marker technology has gained the interest of plant breeders to improve crop plants against complex traits. Molecular markers can be employed to identify the exact location of the genomic region/ QTL, determining the trait for nutritional quality. Once identified, these QTLs can be introgressed to elite cultivars/ varieties. QTL mapping based on biparental mapping populations is found to be less significant. However, genome wide-association mapping studies (GWAS) utilized the diverse germplasm which offers a large number of variations. The extent of linkage disequilibrium determines the marker-trait association. The identified markers linked to the target trait can be utilized in the breeding program to improve the crop plants. The overview of crop improvement based on genomic regions/ QTLs is denoted in Fig. 14.1.

## 14.3 QTLs for pro-Vitamin A

Deficiency of vitamin A is responsible for irreversible loss of vision and is one of the serious health issues in developing nations. It has been reported that over 30% of children and 19 million pregnant women are facing the problem of vitamin A deficiency in developing nations (Duo et al. 2021). Two alleles, viz. *β-carotene hydroxylase 1* (*crtRB1*) and *lycopene epsilon cyclase* (*lcyE*), have been identified which favors pro-vitamin A biosynthesis in maize (Muthusamy et al. 2015). This has gained the interest of researchers to identify QTLs related to pro-vitamin A content in maize. Maize is an important cereal and consumed across the globe. Several QTLs for vitamin A content have been identified in maize (Table 14.1) (Babu et al. 2013; Azmach et al. 2013).

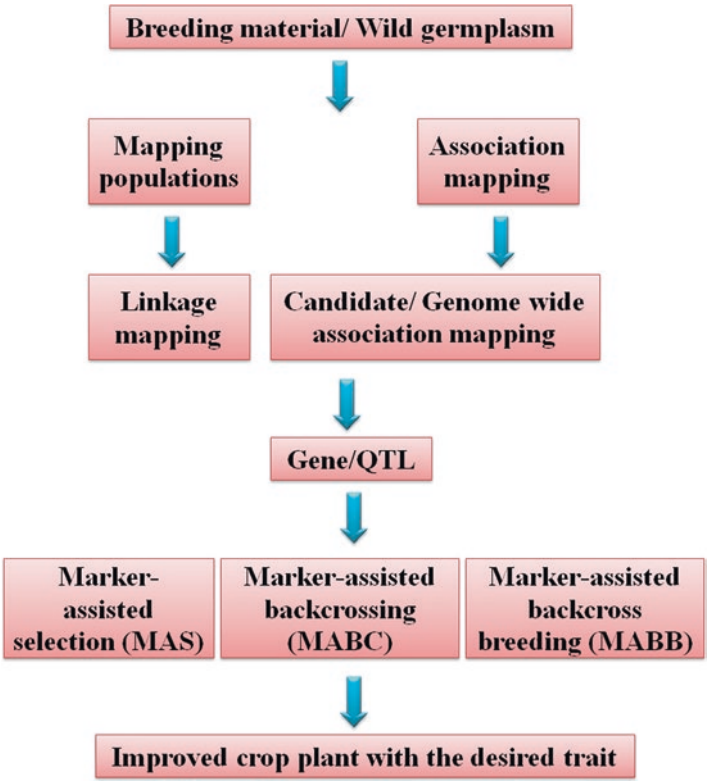


**Fig. 14.1** Schematic representation of QTL-mediated improvement of crop plants

## 14.4 QTLs for Iron (Fe) and Zinc (Zn) Content

Cereals consumed as food are deficient in various micronutrients including Fe and Zn (White and Broadley 2005). As per WHO reports, 30% of the global population are suffering from anemia. To overcome this problem, there is a need to develop cereals with higher Fe and Zn content. In the past few years, several mapping populations have been developed to identify QTLs associated with Fe and Zn content (Table 14.1). In wheat, QTLs for Fe and Zn content have been reported by several authors (Hao et al. 2014; Srinivasa et al. 2014; Tiwari et al. 2016; Crespo-Herrera et al. 2016; Velu et al. 2017, 2018; Gorafi et al. 2018; Liu et al. 2019). Similarly, in barley, QTLs for Fe and Zn were identified on chromosomes 6 and 2 (Mamo et al. 2014; Sadeghzadeh et al. 2015). In rice, several QTLs for Fe and Zn have been mapped on almost all chromosomes. Recently, two QTLs for Fe ($qFe_{9.1}$ and $qFe_{12.1}$) and four QTLs for Zn ($qZn_{1.1}$, $qZn_{5.1}$, $qZn_{9.1}$, and $qZn_{12.1}$) content were identified in double haploid rice (Calayugan et al. 2020). Novel QTLs, qFe3.3 and qFe7.3 for Fe content and qZn2.2, qZn8.3, and qZn12.3 for Zn content, were identified using association mapping (Pradhan

**Table 14.1** List of genomic regions/ QTLs for grain quality in cereals

| Trait | Genomic region/QTLs | Chromosome | Crop | References |
|---|---|---|---|---|
| Vitamin A | *LcyE5′TE, LcyE3′Indel, rtRB1–3′T,* | 10 | Maize | Babu et al. (2013) |
| | *PSY1, lcyE, crtRB1* | 10 | | Azmach et al. (2013) |
| Fe and Zn content | *QGzncpk.cimmyt_2BL* | 2BL | Wheat | |
| | *QZn.bhu_2B, QZn.bhu_6A, and QFe.bhu_3B* | 2B, 6A, 3B | | Srinivasa et al. (2014) |
| | *QZn.bhu_2B, QFe.bhu_2B* | 2B | | Tiwari et al. (2016) |
| | *QZn.Across_4BS, QFe. Across7DS* | 4BS, 7DS | | Crespo-Herrera et al. (2016) |
| | *QGzn.ada_1B, QGzn.sar_1B, QGFe.ada_2B* | 1B, 2B | | Velu et al. (2017) |
| | *QGFe.iari-2A, QGFe.iari-5A, QGFe.iari-7A and QGFe.iari-7B, QGZn.iari-2A, QGZn.iari-4A, QGZn.iari-5A, QGZn.iari-7A and QGZn.iari-7B* | 2A, 4A, 5A, 7A, 7B | | Krishnappa et al. (2017) |
| | *QZn 2A, QZn7B* | 2A, 7B | | Velu et al. (2018) |
| | *qFes1, qfes2, qZns1, qZns2* | 4D, 2D, 5D, 1D | | Gorafi et al. (2018) |
| | *QGZn.co-5A, QGZn.co-7A, QGFe.co-3B.1, QGFe.co-5A.2* | 5A, 7A, 3B | | Liu et al. (2019) |
| | *QZn.caas-1DS, QZn.caas-2AS, QZn.caas-3BS, QZn.caas-4DS, QZn.caas-6AS, QZn.caas-6DL, QZn.caas-7BL, QFe.caas-3BL, QFe.caas-4DS, QFe.caas-6AS, QFe.caas-7BL* | 1DS, 2AS, 3BS, 4DS, 6AS, 6DL, 7BL, 3BL | | Wang et al. (2021a, b) |
| | *Zn-qtl-6H_ SCRI_RS_10655* | 6HL | Barley | Mamo et al. (2014) |
| | *QTL.Zn* | 2HS, 2HL | | Sadeghzadeh et al. (2015) |
| | *Fe-2H-84.74, Fe-2H-139.62, Fe-4H-67.9, Fe-1H-54.5, Fe-1H-57.85, Fe-1H-90.04, Fe-2H-84.74, Fe-4H-53.87, E-4H-54.95, Fe-6H-102.03, Fe-7H-17.62, Zn-2H-87.34, Zn-1H-21.97, Zn-2H-148.16, Zn-2H-40.12, Zn-2H-86.84* | 2H, 4H, 1H, 4H, 6H, 7H | | Gyawali et al. (2017) |
| | $qFe_2$, $qZn_5$ | 2, 5 | Rice | Zhang et al. (2014) |
| | $qFe_{1.2}$ *(gene OsYSL1),* $qFe_{5.1}$ *(gene OsZIP6),* $qFe_{7.2}$ *(gene OsZIP8)* | 1, 5, 7 | | Agarwal et al. (2014) |
| | $qFe_6$, $qZn_8$ | 6, 8 | | Xu et al. (2015) |

(continued)

**Table 14.1** (continued)

| Trait | Genomic region/QTLs | Chromosome | Crop | References |
|---|---|---|---|---|
| | *$qFe_{10.1}$, $qZn_{6.2}$, $qZn_{7.1}$* | 10, 6, 7 | | Descalsota et al. (2018) |
| | *$qFe_{1.2}$, $qFe_{11.1}$, $qZn_{2.1}$, $qZn_{3.2}$, $qFe_{3.2}$, $qFe_{4.1}$, $qZn_{5.1}$, $qZn_{12.1}$* | 1, 11, 2, 3, 4, 5, 12 | | Swamy et al. (2018) |
| | *$qFe_{1.1}$ $qFe_{1.2}$, $qZn_{1.1}$, $qFe_{6.1}$, $qZn_{6.1}$, $qFe_{6.2}$, $qZn_{6.2}$* | 1, 6 | | Dixit et al. (2019) |
| | *QTL.Fe.$_9$, QTL.Zn.$_4$* | 9, 4 | | Islam et al. (2020) |
| | *$qFe_{3.3}$, $qFe_{7.3}$, $qZn_{2.2}$, $qZn_{8.3}$, $qZn_{12.3}$* | 3, 8, 12 | | Pradhan et al. (2020) |
| | *$qFe_{9.1}$, $qFe_{12.1}$, $qZn_{1.1}$, $qZn_{5.1}$, $qZn_{9.1}$, $qZn_{12.1}$* | 9, 12, 1, 5 | | Calayugan et al. (2020) |
| | *$qFe_7$, $qZn_7$* | 7 | | Jeong et al. (2020) |
| | *qZPR.$_{1.1}$, qZPR.$_{11.1}$* | 1, 11 | | Suman et al. (2021) |
| | *Fe, Zn* | LG 3, 5, 7 | Pearl millet | Kumar et al. (2016) |
| | *Fe, Zn* | LG 3, 5, 7 | | Anuradha et al. (2017) |
| | *qFe1/54 and qZn1/54* | LG 1, 7 | | Kumar et al. (2018) |
| | *PglZIP, PglNRAMP, PglFER* (gene families) | LG 7 | | Mahendrakar et al. (2020) |
| | *Fe, Zn* | Pgl01, Pgl02, Pgl04, Pgl05, Pgl06 (2), Pgl07 | | Pujar et al. (2020) |
| | *QFe2.1, QFe2.1, QFe3.1, QFe5.1, QFe7.1, QZn2.1, QZn3.1, QZn3.2, QZn6.1,* | LG 1, 2, 3, 5, 6, 7 | | Singhal et al. (2021) |
| Amino acids and GPC | *QPro.mgb-4B, QPro.mgb-5A, QPro.mgb-6A.1, QPro.mgb.6A.2, QPro.mgb.6B, QPro.mgb-7A, QPro.mgb-7B* | 4B, 5A, 6A, 6B, 7A, 7B | Wheat | Blanco et al. (2002) |
| | *GPC* | 2AS, 6AS and 7BL | | Blanco et al. (2006) |
| | *QGpc.sdau-3B, QGpc.sdau-5A, QGpc.sdau-6A* | 3B, 5A, 6A | | Sun et al. (2008) |
| | *QGpc\tgw.WL-1D, QGpc.WL-2A, QGpc\yld.WL-2B, QGpc.WL-3B, QGpc.WL-4A, QGpc\yld.WL-4A, QGpc.WL-5B, QGpc.WL-5D, QGpc.WL-6B, QGpc.WL-7A* | 1D, 2A, 2B, 3B, 4A, 5B, 5D, 6B, 7A | | Wang et al. (2012) |
| | *QGPC.bhu_1A* | 1A | | Tiwari et al. (2016) |
| | GPC, Protein yield | 1A, 1B, 2A, 2D, 3A-1, 3A-2, 3B, 3D-2, 4A, 4B, 5A, 5B, 5D, 6A, 7A-1, 7B | | Mahjourimajd et al. (2016) |

(continued)

**Table 14.1** (continued)

| Trait | Genomic region/QTLs | Chromosome | Crop | References |
|---|---|---|---|---|
| | *QGpc.sdau-1D, QGpc.sdau-2D, QGpc.sdau-4A, QGpc.sdau-1A, QGpc.sdau-2A.2, QGpc.sdau-4B, QGpc.sdau-5D, QGpc.sdau-7A, QGpc.sdau-2A.1,* | 1A, 1D, 2A, 2D, 4A, 4B, 5D, 7A | | Sun et al. (2016) |
| | *QGpc.2B-yume* | 2B | | Terasawa et al. (2016) |
| | *QGpc.uhw-4B, QGpc.uhw-5A.1, QGpc.uhw-6B, QGpc.uhw-7B.2* | 4B, 5A, 6B, 7B | | Fatiukha et al. (2020) |
| | *QGpc-1B-2, QGpc-4B-1.4* | 1B, 4B | | Guo et al. (2020) |
| | *QGPC.cib-4A* | 4A | | Li et al. (2020) |
| | PC | 6, 7 | Rice | Tan et al. (2001) |
| | *pro1* | 1 | | Aluko et al. (2004) |
| | *qCP-12* | 12 | | Zhang et al. (2008) |
| | *qPC11.2* | 11 | | Qin et al. (2009) |
| | *qPC-3, qPC-4, qPC-5, qPC-6, qPC-10* | 3, 4, 5, 6, 10 | | Yu et al. (2009) |
| | *qAa1, qAa7, qAa9* | 1, 7, 9 | | Zhong et al. (2011) |
| | *qPro-2, qPro-10* | 2, 10 | | Yun et al. (2014) |
| | *qPro-2* | 2 | | Lee et al. (2014) |
| | *qPC1* | 1 | | Peng et al. (2014) |
| | *qPC-1* | 1 | | Yang et al. (2015) |
| | *qPC6.2* | 6 | | Kinoshita et al. (2017) |
| | *qGPC1.1, qSGPC2.1, qSGPC7.1* | 1, 2, 7 | | Chattopadhyay et al. (2019) |
| | *qPC3.1, qPC5.1, qPC9.1* | 3, 5, 9 | | Pradhan et al. (2019) |
| | *QTL.pro.1* | 1 | | Islam et al. (2020) |
| | *qAAC6.1, qAAC7.1, qPC1.2* | 1, 6, 7 | | Jang et al. (2020) |
| | *qGPC1–1* | 1 | | Wu et al. (2020) |
| | *PC* | 6H | Barley | See et al. (2002) |
| | *GPC* | 2, 6 | | Mickelson et al. (2003) |
| | *GPC* | 2H, 4H, 5H 7H | | Emebiri et al. (2003) |
| | *Qgpc1H, Qgpc2H, Qgpc4H, Qgpc5Ha, Qgpc5Hb, Qgpc5Hc, Qgpc7H* | 1H, 2H, 4H, 5H, 7H | | Emebiri et al. (2005) |
| | *Qcp2a, Qcp3a, Qcp5a, Qcp6a, Qcp7a, Qcp7b* | 2H, 3H, 5H, 6H, 7H | | Abdel-Haleem et al. (2010) |
| | *QGpc.ZgSc-2H.1, QGpc. ZgSc-2H.2, QGpc.ZgSc-2H.3, QGpc.ZgSc-4H.1, QGpc. ZgSc-4H.2, QGpc.ZgSc-4H.3, QGpc.ZgSc-5H.3, QGpc. ZgSc-5H.1, QGpc.ZgSc-5H.2, QGpc.ZiSc-7H.1, QGpc. ZiSc-7H.2, QGpc.ZiSc-7H.3* | 2H, 4H, 5H, 7H | | Fan et al. (2017) |

et al. 2020). Pearl millet is a nutrient-rich cereal; QTLs/ candidate genes for Fe and Zn content were identified on linkage group (LG) 3 and 5 (Kumar et al. 2016), LG 3, 5, and 7 (Anuradha et al. 2017), LG 1 and 7 (Kumar et al. 2018), LG 7 (Mahendrakar et al. 2020), Pgl01, Pgl02, Pgl04, Pgl05, Pgl06 (2), and Pgl07 (Pujar et al. 2020), and LG 2 and 3 (Singhal et al. 2021).

## 14.5 QTLs for Amino Acids and Grain Protein Content

Grain protein content (GPC) of cereals is an important component of human diet and is the determining factor of nutritional quality of plant-based diet. In the past few years, several researchers have mapped QTLs for GPC in both durum and hexaploid wheat (Table 14.1) (Blanco et al. 2002, 2006; Mahjourimajd et al. 2016; Sun et al. 2016; Fatiukha et al. 2020). In rice, QTLs for GPC have been mapped on all the chromosomes. However, most of them are located on chromosomes no 1, 2, 6, 7, 10, and 11 (Tan et al. 2001; Aluko et al. 2004; Zhang et al. 2008; Qin et al. 2009; Yu et al. 2009; Zhong et al. 2011; Lee et al. 2014; Yun et al. 2014; Yang et al. 2015; Chattopadhyay et al. 2019; Pradhan et al. 2019; Jang et al. 2020; Wu et al. 2020). Similarly, in the case of barley, QTLs for GPC have been reported on all seven chromosomes by several researchers. It has been found that seven consensus QTLs are present on chromosomes 2H, 4H, 5H, 6H, and 7H (See et al. 2002; Mickelson et al. 2003; Emebiri et al. 2003, 2005; Abdel-Haleem et al. 2010; Fan et al. 2017).

## 14.6 Commercial Varieties with Improved Nutritional Value

Once the QTLs get identified, marker-assisted breeding (MAB) can be employed to develop improved versions of commercial hybrids/ cultivars. This started with the development of improved version of commercial pearl millet hybrid against downy mildew (Hash et al. 2006). Thereafter, marker-assisted selection (MAS) has been widely adopted to improve cereals like rice (Improved Pusa Basmati 1, Improved Samba Mahsuri, Swarna sub1, IR64 sub1, PRR78/IRBB60, Pusa 6A, and Improved Pusa RH10), wheat, and maize. Various commercial hybrids/ cultivars of rice have been improved against bacterial blight, submergence tolerance, rice blast, drought tolerance, and several other traits (Kottapalli et al. 2010; Reddy et al. 2009; Singh et al. 2011; Das et al. 2017). Similarly, wheat varieties (Patwin, Expresso, Lassik, Farnum, Westmore, and AGS2026) have been improved against various biotic and abiotic stresses using MAS (Gupta et al. 2010). Several attempts have been made to improve the nutritional value of cereals using MAS. One of the success stories in cereals is development of quality protein maize (QPM) (Vivek et al. 2008). Marker-assisted backcrossing (MABC) was utilized to introgress the Opaque 2 allele on chromosome 2 and led to the

release of 'Vivek-QPM-9' in 2008 (Gupta et al. 2013). The improved hybrid possessed 41% more tryptophan and 30% more lysine than the original hybrid (Vivek Hybrid 9). Later on in the year 2017, improved versions of three popular hybrids, viz. Pusa HM-4, Pusa HM-8, and Pusa HM-9, were released for commercial cultivation in India (Hossain et al. 2018). MABC has been utilized to develop biofortified rice with higher Zn content. With the efforts of BRRI (Bangladesh Rice Research Institute), five high Zn content varieties, viz., BRRI dhan62, BRRI dhan64, BRRI dhan72, BRRI dhan74, and BRRI dhan84, have been released for commercial cultivation. Similarly, high-Zn varieties, DRR Dhan45 and Chhattisgarh Zinc Rice-1 in India, NSIC Rc 460 in Indonesia, and Nutri Zn in Philippines, have been commercialized (Calayugan et al. 2021). In 2017, two Fe and Zn biofortified varieties, WB 02 and HPBW 01, have also been released for commercial cultivation by the Indian Institute of Wheat and Barley Research (Yadava et al. 2020). In the same year, two Fe and Zn biofortified hybrids of pearl millet, viz. AHB 1200 and HHB 299, were released with the combined efforts of CCS Haryana Agricultural University, Hisar and ICRISAT, Hyderabad (Yadava et al. 2020).

Over the past decade, the MAB has been widely adopted to develop improved cereals. However, the global population is rising at an alarming rate and is projected to touch the mark of 9 billion by 2050 than its present level 7.53 billion (Priti et al. 2018). To keep pace with the rising population, researchers need to develop healthier and high-yielding cereals. The present, modern, and conventional agricultural practices are not capable of meeting such high demands. There is a need for newer technology that is fast with higher precision rate. GE is one such technology which can be utilized to its full potential to meet the global demands.

## 14.7 Genome Editing (GE)

The existing modern breeding technologies need to be supplemented with advanced techniques like GE to develop nutrient-rich cereals. The emergence of GE technology has revolutionized the cereal improvement program with its superior precision rate and speed (Matres et al. 2021). GE allows site-specific modification of DNA. This offers significant advantage over GM technology that mostly relied on random integration of introgressed DNA. Since the development of GE in 2010, it has been consolidated into three major platforms, viz., ZNFs, TALENs, and CRISPR-Cas. These use designed nucleases to introduce targeted DSBs. These breaks are repaired in an error-prone pathway and mutations are introduced within the target gene. These genetic changes have been utilized for crop improvement (Christou et al. 2021). The utilization of the three above-mentioned GE platforms for grain quality improvement in cereals is discussed below.

## 14.8 Zinc Finger Nucleases (ZFNs)

In recent years, new advances empowering targeted alteration of plant genomes have been made possible and new genome editing technologies have been developed including ZFNs (Bibikova et al. 2002). ZFNs are a class of designed restriction enzymes that proves to be a powerful means for GE. ZFNs are chimeric proteins that consist of an N-terminal DNA-binding domain and a nonspecific DNA cleavage domain. Each designed zinc finger (ZF) identifies a specific 3-bp DNA sequence by binding and a FokI nuclease. FokI space dimerization is profoundly a basic requirement for ZFN enzymatic action (Kim et al. 1996). FokI belongs to the type IIS class of restriction endonucleases. The capacity of these designed nucleases to make targeted double-stranded breaks at assigned locations throughout the genome has enabled the editing of genes with great precision. A couple of Zinc finger arrays (30 amino acid residues) exhibit ties to respective sequences and get aligned in contrary style with one another resulting in a particular configuration (Petolino 2015). The binding sites of two Zinc finger arrays are 18–24 bp in length separated by 5–8 bp. This space is important for creating DSB in the target sequence. The DSBs introduced by these endonucleases are followed by error-prone nonhomologous end joining (NHEJ) repair results in deletions or insertions, base substitutions, or incorporation of exogenous donor sequences at the ZFN cleavage site. If this damaged repair is in the coding region of a gene, it can interrupt the reading frame resulting in an inactive gene creating new genetic variations either by addition or deletion. The mechanism of action of ZFNs is represented in Fig. 14.2. ZFNs were first reported in Arabidopsis (Wright et al. 2005; Llyod et al. 2005) and tobacco (Cai et al. 2008; Maeder et al. 2008; Townsend et al. 2009), and later on, effectively used in different plants such as soybean (Curtin et al. 2011) and rice (Cantos et al. 2014; Jung et al. 2018).

One of the most significant examples of GE for grain quality improvement by ZFNs targeted the *IPK1* gene which encodes inositol-1,3,4,5,6-pentakiphosphate 2–kinase, which is an important enzyme in phytate biosynthesis in maize seeds (Shukla et al. 2009), resulting in herbicide tolerance and altered inositol phosphate profiles in the developing seeds. Starch biosynthesis pathway was targeted in rice with engineered ZFNs which can effectively cleave and induce mutations at SSIVa locus. This modification resulted in dwarfism and reduced starch content (Jung et al. 2018).

The field of GE has been revolutionized by Zinc finger nucleases, showing the ability to utilize genomic sites of concern and opened the entryways for both essential and applied research. As for productivity, ZFNs give an advantage over different tools, extraordinary explicitness, and insignificant nontarget impacts and present challenges are focused on further improving plan and conveyance just as expanding their utilization in different crops. The advances in ZFN-based GE give huge freedom to focus on any DNA sequence in the genome. However, there are a few intrinsic detriments that have confined their wider scope of utilization, for example, costly and tedious cycles for enhanced assembly of the ZF domains and off-target effects (Eid and Mahfouz 2016; Mushtaq et al. 2019).

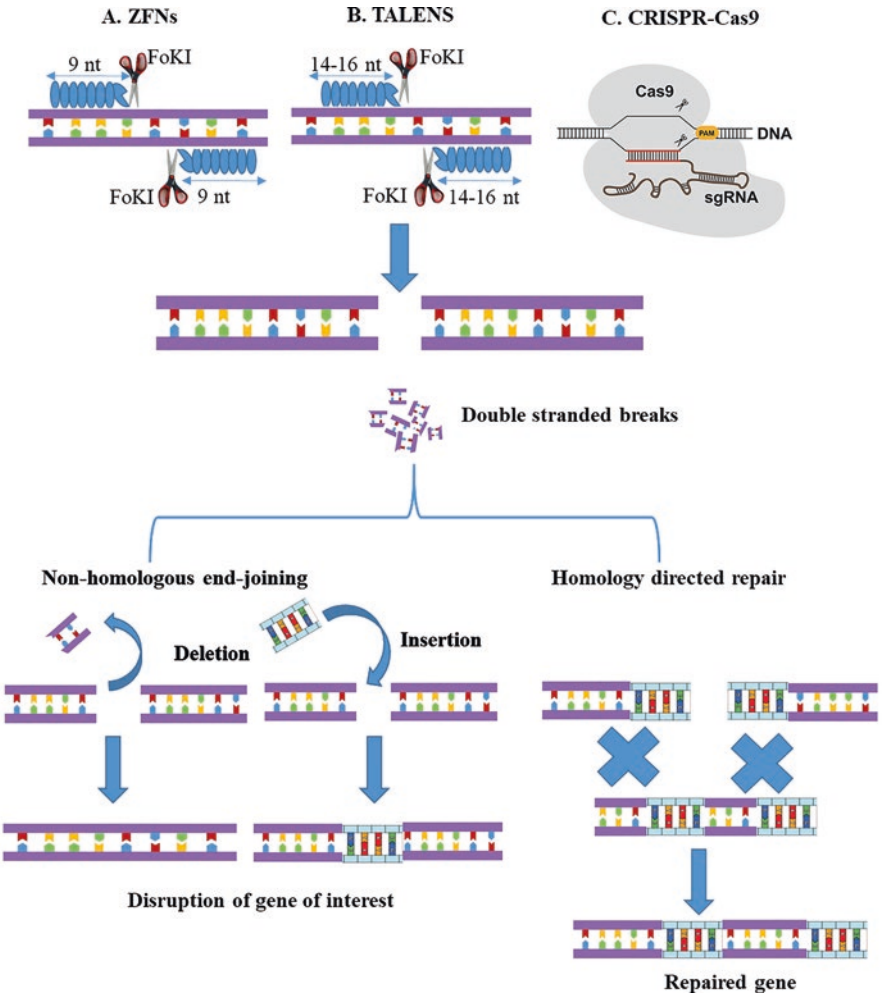


**Fig. 14.2** An overview of nonhomologous end joining (NHEJ) and homology-directed repair (HDR)-mediated genome editing

## 14.9 TALENs

Precise genetic editing or modification has remained the fundamental goal for researchers engaged in the fields of molecular breeding and agricultural biotechnology. Engineered nucleases are dynamic tools for precise in vivo genetic modifications in genomes (Bogdanove and Voytas 2011). TALENs promptly turn up as an alternative to ZFNs, by introducing targeted DSBs for GE, and provide a novel and excellent route for crop improvement. TALENs and ZFNs have similar features of containing FokI nuclease domain combined with customizable DNA-binding

domain (Fig. 14.2). This DNA-binding domain is comprised of highly conserved repeats which are derived from transcription activator-like effector proteins (TALEs), which are fused with nonspecific cleavage domain of FokI endonucleases. TALEs are Type III effector proteins that were discovered in plant pathogenic bacteria *Xanthomonas* spp. from rice and cotton (Boch and Bonas 2010). Similar to ZFNs, TALENs facilitate specific GE via induction of DSB in a specific target sequence of genome, followed by nonhomologous end joining (NHEJ) (Moehle et al. 2007) or homology-directed repair (HDR) (Rémy et al. 2010). The emergence of engineered nucleases has revolutionized the field of genetic engineering and TALENs use these engineered nucleases to introduce specific additions and deletions in targeted genes. TALEN assembly comprises central DNA-binding domain, highly conserved acidic transcription activation domain at C terminal, secretion, and translocation at N terminal and nuclear localization signal (NLS). In central DNA-binding domain, 33–35 amino acids tandem repeats are present which recognizes one nucleotide in the target sequence (Li et al. 2011). The specificity of TALEN mainly depends on amino acids of polymorphic nature located at 12 and 13 positions, also called repeat variable di-residue (RVD). Many RVDs have been reported; some major ones are NI (Asn ile), HD (His Asp), NN (Asn Asn), and NG (Asn Gly), which recognize nucleotides adenine (A), cytosine (C), guanine (G), thymine (T), respectively. These tandem repeats are followed by a sequence of 20 amino acids, which are called half repeats.

TALENs have been used widely for crop improvement for engineering disease resistance and increasing shelf life (Haun et al. 2014; Wang et al. 2014; Wendt et al. 2013; Lor et al. 2014). TALENs have also been used for grain quality improvement in cereal crops being utmost priority for sustainable agriculture (Table 14.2). In a study by Ma et al. (2015), the storage tolerance of rice was increased by knocking

**Table 14.2** Overview of various crop plants improved for grain quality using GE

| Sr. No. | Crop | Gene | Trait | References |
|---|---|---|---|---|
| **ZFNs** | | | | |
| 1. | Maize | *IPK1* | Phytic acid | Shukla et al. (2009) |
| 2. | Rice | *SSIVa* locus | Starch content | Jung et al. (2018) |
| **TALENS** | | | | |
| 1. | Rice | *LOX3* | Storage | Ma et al. (2015) |
| 2. | Rice | *OsBADH2* | Fragrance | Shan et al. (2015) |
| **CRISPR-Cas** | | | | |
| 1. | Maize | *ZmIPK1* | Phytic acid | Sun et al. (2007) |
| 2. | Maize | *ZmIPK1* | Phytic acid | Liang et al. (2014) |
| 3. | Maize | *ZmMADS47* | Protein content | Qi et al. (2016) |
| 4. | Maize | *Wx1* | Starch content | Waltz (2016) |

(continued)

**Table 14.2** (continued)

| Sr. No. | Crop | Gene | Trait | References |
|---|---|---|---|---|
| 5. | Maize | *Wx1* | Starch content | Qi et al. (2020) |
| 6. | Maize | *Wx1* | Starch content | Gao et al. (2020) |
| 7. | Maize | *BADH2* | Fragrance | Wang et al. (2021a, b) |
| 8. | Rice | *OsFAD2–1* | RBO | Abe et al. (2018) |
| 9. | Rice | *OsFAD2* | RBO | Bahariah et al. (2021) |
| 10. | Rice | *BADH2* | Fragrance | Shao et al. (2017) |
| 11. | Rice | *BADH2* | Fragrance | Fuhua et al. (2018) |
| 12. | Rice | *BADH2* | Fragrance | Usman et al. (2020) |
| 13. | Rice | *BADH2* | Fragrance | Ashokkumar et al. (2020) |
| 14. | Rice | *Osor* | Vitamin A | Endo et al. (2019) |
| 15. | Rice | *Osor* | Vitamin A | Dong et al. (2020) |
| 16. | Rice | *Wx* | Amylose content | Zhang et al. (2018a, b) |
| 17. | Rice | *Wx* | Amylose content | Yunyan et al. (2019) |
| 18. | Rice | *Wx* | Amylose content | Li et al. (2020a) |
| 19. | Rice | *SBEI* and *SBEII* | Amylose content | Sun et al. (2017) |
| 20. | Rice | *SBEII* | Amylose content | Baysal et al. (2020) |
| 21. | Rice | *Rc* | Proanthocyanidins and anthocyanins | Zhu et al. (2019) |
| 22. | Wheat | α-Gliadin | Gluten content | Sánchez-León et al. (2018) |
| 23. | Wheat | α-And γ-gliadin | Gluten content | Jouanin et al. (2019) |
| 24. | Wheat | α-Gliadin | Gluten content | Sánchez-León et al. (2018) |
| 25. | Wheat | *Pinb, waxy,* and *DA1* | Grain hardness, starch quality, and kernel size | Zhang et al. (2018a, b) |
| 26. | Wheat | α-Amylase/trypsin inhibitors | Protein quality | Camerlengo et al. (2020) |
| 27. | Wheat | *TaSBEIIa* | Amylose content | Li et al. (2020b) |
| 28. | Wheat | *Pinb, waxy, ppo* and *psy* | Grain hardness, starch quality, and dough color | Zhang et al. (2021) |
| 29. | Barley | *HvPAPhy_a* | Phytic acid | Holme et al. (2017) |
| 30. | Barley | *HvITPK1* | Phytic acid | Vlčko and Ohnoutková (2020) |
| 31. | Barley | D-hordein | Glutenins | Yang et al. (2020) |
| 32. | Sorghum | *k1C* | Kafirins | Li et al. (2018) |

out *LOX3* gene. Fragrant rice is favored over non-fragrant rice all over the world. In fragrant rice, more than a hundred volatile compounds are found, one of which is 2-acetyl-1-pyrroline (2AP). Its quantity is higher in fragrant rice than non-fragrant rice. Shan et al. (2015) employed TALENs to disrupt *OsBADH2* gene which inhibited the synthesis of 2AP. The study showed that the 2AP content of non-fragrant rice increased from 0 to 0.35–0.75 mg/kg, which was almost similar to the positive control variety with mutation in *BADH2* gene.

## 14.10 CRISPR/ Cas9

The combination of research and technology in developing improved genotypes of crop plants has led to the basis of modern agriculture. Though nowadays traditional breeding is much faster than 50 years back, it is not able to cope with the increasing food demand with the global climate change making the situation more challenging. As far as crop improvement is dependent on conventional breeding, i.e., exploitation of natural germplasm variation and introgressing the desired trait in target crops, time and resources will always limit the crop improvement (Jangra et al. 2017, 2019a, b). These limitations can be overcome by exploiting CRISPR technologies and crop improvement can be accelerated at a rate that was not possible earlier. CRISPR in agriculture can be considered as a novel breeding method that is much faster, predictable, and cheaper and the results are identical to conventional breeding (Gao et al. 2018). Since its first report in 2012, this technology has revolutionized research in life sciences.

Initially, CRISPR was identified as repeats (Ishino et al. 1987) and was later characterized in 1990s. The term CRISPR was coined by Jansen et al. (2002). It is a bacterial and archaeal defense mechanism that provides immunity against bacteriophages through RNA-programmed DNA cleavage. The Cas9 system is composed of a cascade of different proteins generally classified into two classes according to the structure, 6 type and 19 subtype (Shmakov et al. 2017). The composition of effector nucleases determines the level of variation among the classes. The class I effectors comprise of a complex of several proteins with different functions; however, the class II effector comprises a multi-domain single protein (Makarova et al. 2015). The most used CRISPR is type II-A CRISPR/Cas9 system, and due to its high efficiency in producing double-stranded breaks, the spCas9 is derived from *Streptococcus pyogenesis*. Some restrictions like proto-spacer adjacent motif (PAM) were showed by spCas9. PAM is NGG (N-Any nucleotide, G-Guanine), making its application difficult in sequences having higher AT and also prone to produce off-target effects. These limitations have been overcome by high fidelity variants of Cas9 with mutations that prevent nonspecific interactions between DNA and nuclease domains leading to reduced off-target effects (Kleinstiver et al. 2016). Expression, interference, and adaptation are the three stages of which the CRISPR/Cas9 system is comprised. In the expression of CRISPR array, sequences that are homologous to the target sequence (proto-spacers) get transcribed into pre-CRISPR

RNA (pre-crRNA). Homologous bonds with trans activating crRNA (tracrRNA) are formed by these pre-crRNAs. After the formation of pre-crRNA/tracrRNA complex, the Cas9 protein gets attached and RNase III cut the long pre-cdRNAs into separate crRNA/tracrRNA complexes (gRNA). Interference starts with guiding of Cas9 complex by crRNA/tracrRNA to target sequence and gRNA binds to target sequence after PAM. As the PAM sequence is not present in CRISPR array, PAM allows the discrimination between self/nonself. The target sequence, unwound as Cas9, is equipped with helicase and nuclease activity and the cuts are produced by the RuvC and HNN domain of Cas9, leading to DSB in the target sequence. NHEJ or HDR repairs the DSB and the repaired sequence is transcribed and adapted into the genome as described in Fig. 14.2 (Jackson et al. 2017). CSISPR/Cas9 offers several advantages over other GE technologies being simple and cost-effective. Also, the Cas9 system is readily available making it a highly valuable GE tool. The multi-target approach of this technique could be utilized to target multiple genes simultaneously. The off-target effects of the Cas9 could be reduced by mutating RuvC domain.

CRISPR allows researchers to perform gene knockout, DNA-free gene editing, gene insertions or knock-ins, and transient gene silencing. In the case of gene knockout/ gene silencing, CRISPR utilizes a single guide RNA (sgRNA) to initiate double-stranded breaks at the target site using Cas9 endonuclease. The repairing of these breaks through NHEJ mechanism (error-prone) results in genomic deletions or insertions, leading to permanent silencing of target gene. The DNA vector-free CRISPR-based GE requires only RNA or protein components. This DNA-free editing eliminates the possibility of unwanted genetic alterations that may be caused due to integrating plasmid DNA or random vector integration at the cut site. The double-stranded breaks induced through CRISPR can be utilized for creating gene 'knock-ins' through homology-mediated repair. The gene codon can be altered by the precise addition of donor template. In earlier studies, it has been found that precise insertions can be made by CRISPR-Cas9 system with the help of single-stranded DNA (Cong et al. 2013). Transient gene silencing or suppression of transcription can be done with modified Cas9 which is unable to cut DNA. The promoter region is targeted by the modified Cas9 and the transcriptional and gene expression activity is hampered. This can also be utilized for transient activation or upregulation of target genes (Ishino et al. 2018).

An efficient CRISPR-Cas delivery system is the only prerequisite for the application of this technology in crop improvement. The transformation of major crop plants is generally confined to a few genotypes per species, which are not probably the elite cultivars. Therefore, the development of user-friendly and robust CRISPR delivery system for commercial varieties is essential. A recent study showed that the transformation efficiency of cereals can be improved with the help of morphogenic regulators (Lowe et al. 2016). The transformation of recalcitrant elite cultivars of wheat and corn can be improved by haploid-inducer-mediated GE approach (Kelliher et al. 2019; Wang et al. 2019). The time-taking process and labor-intensive process of plant regeneration through tissue culture after transformation could be avoided by

administrating the CRISPR components to shoot apical meristem, pollen, or flowering tissues (Hickey et al. 2019). This tissue culture-free technique has been recently employed to develop gene-edited plants by de novo meristem induction (Maher et al. 2020). Though in eukaryotes NHEJ is the major mechanism involved in DNA repair in crops, many desired traits can be attained by specific substitution or insertion of DNA segments. A novel method of base substitution is provided by base editing; however, it is currently restricted to A-G or C-T substitutions (Komor et al. 2016; Gaudelli et al. 2017). In a recent development, a ground-breaking genome editor called 'prime editing' has been developed that delivers genetic information directly into specific DNA sites providing a powerful tool to expand the scope and capabilities of GE (Anzalone et al. 2019). In prime editing, the Cas9 is engineered to function as nickase combined with reverse transcriptase, and the sgRNA is replaced with pegRNA (prime editing guide RNA), which comprises both a sgRNA for target site identification and RNA template for determining the DNA sequences that are to be integrated at the target site (Anzalone et al. 2019). Similar applications may follow in crop plants in the not-so-distant future.

Since the recognition of CRISPR as cutting-edge technology, it has gained the interest of researchers and industries to improve major crop plants. In a very short time since its first application in plants, it has been utilized to improve various traits like tolerance against biotic and abiotic stresses, quality, nutritional value (Table 14.2), and yield (Arora and Narula 2017; Jaganathan et al. 2018; Gao et al. 2018; Wang et al. 2019; Ahmad et al. 2020; Zaidi et al. 2020; Zhang et al. 2020; Zhang et al. 2021). At present, CRISPR/Cas has become a major biotechnology tool to introgress the desired trait.

## 14.11 CRISPR/Cas for Grain Quality Improvement in Cereals

CRISPR/Cas has been widely employed to edit quality-related genes in the case of cereals to enhance their nutritional value. Maize, rice, wheat, barley, oats, rye, and sorghum are the principal cereal crops across the globe. In the following section, an overview of CRISPR-Cas9-based improvement of grain nutrient quality in cereals is summarized in Table 14.2.

## 14.12 Maize (*Zea Mays*)

Maize is the most cultivated cereal all across the globe. It is widely used for human consumption, animal feed, and biofuel production. Phytic acid (inositol 1, 2, 3, 4, 5, 6-hexakisphosphate) is an anti-nutritional compound present in maize and reduces

the assimilation of minerals after human and animal consumption (Feil 2001). To overcome this problem, the gene (Inositol phosphatase kinase 1, *ZmIPK1*) encoding for phytic acid production was knocked out using CRISPR-Cas9. The study showed that over 50% of the IPK1 open reading frames were interrupted in leaves and seeds (Sun et al. 2007). Similarly, ZmIPK1 was targeted, knocked out by mutagenesis induced by specifically designed gsRNA (Liang et al. 2014).

Zeins are the most abundant storage protein in maize and are deficient in two essential amino acids (lysine and tryptophan), contributing to poor nutrient quality. Opaque 2 (O2), a basic leucine zipper protein-based transcription factor, regulates the synthesis of zeins in maize (Schmidt et al. 1990). It has been reported that this problem can be overcome by altering the zeins production, which allows production of other proteins with higher lysine and tryptophan content. CRISPR technology has been employed to target *ZmMADS47* gene encoding a MADS-box protein, an interacting partner of O2. A reduction of 12.5% in zeins content was recorded in MADS/CAS9–21 lines (Qi et al. 2016).

Starch (amylose and amylopectin) content is another important target trait. Generally, the starch content in normal maize is around 70%, of which 75% is amylopectin and 25% is amylose. Waxy maize that has high amylopectin content was first discovered over 100 years ago in China. The high starch content makes waxy corn an ideal product for implementing CRISPR-Cas to overcome the challenges associated with conventional breeding. CRISPR-Cas technology has been utilized to alter the waxy gene (*Wx1*), which encodes granule-bound starch synthase responsible for amylose production in endosperm. The alteration can lead to accumulation of high amylopectin content in endosperm, making it suitable for various industries like processed foods, adhesives, and high-gloss paper. For commercialization, CRISPR-Cas-based editing of *WX1* has been applied to elite commercial cultivars and crossbreed as CRISPR-waxy hybrids (Waltz 2016). Waxy maize was presented among first CRISPR-edited crops that can be cultivated and sold free from USDA regulations and will be available in the market by DuPont Pioneer in the coming years after field trials (Waltz 2018). In another study, the *Wx1* gene has been targeted to develop waxy maize employing CRISPR-Cas9 (Qi et al. 2020). Field trials of CRISPR-edited waxy corn hybrids of 12 elite inbred lines indicated that these were agronomically superior to the introgressed line and produced on an average 5.5 bushels per acre higher yield (Gao et al. 2020).

Recently, aromatic maize has been developed using CRISPR-Cas9. Two maize betaine aldehyde dehydrogenase 2 (BADH2) homologs, ZmBADH2a and ZmBADH2b, were identified in maize. Zmbadh2a and zmbadh2b single mutants and the zmbadh2a-zmbadh2b double mutant were developed by CRISPR/Cas in four inbred lines. The double mutants accumulated 0.028 and 0.723 mg/kg 2-acetyl-1-pyrroline (2AP) in fresh and dried kernels, respectively (Wang et al. 2021a, b).

## 14.13 Rice (*Oryza Sativa*)

Rice is consumed by more than 3.5 billion people across the globe and accounts for around 20% of the global dietary supply (Fiaz et al. 2019; Ku and Ha 2020). It has been estimated that there should be a 40% increase in production by 2030 to meet the global rice demand (Khush 2005). In addition, with the improvement in people's living standards, the demand for rice with higher nutritional quality is expected to increase. Therefore, newer technologies like CRISPR can be utilized to improve the nutritional value of rice at a much faster rate than conventional breeding. Rice bran oil (RBO) is widely used in Asian countries and the major components of RBO are monounsaturated oleic acid (37–52%) followed by 13–22% of linoleic acid (polyunsaturated) and 27–40% palmitic acid (saturated) (Taira et al. 1988). The presence of oleic acid in RBO makes it good for health which has increased the demands of rice bran oil. Further, increasing the oleic acid in rice can add to the health benefits. In plants, the conversion of oleic acid to linoleic acid is catalyzed by an enzyme fatty acid desaturase 2 (FAD2). In rice, three functional *FAD2* genes are present, of which *OsFAD2–1* is highly expressed in seeds. Thus, by altering this gene, the oleic acid content in RBO can be elevated. The disruption of *OsFAD2–1* gene by targeted mutagenesis utilizing CRISPR led to two-fold increase in oleic acid content, thereby increasing the quality of RBO (Abe et al. 2018). In another study, CRISPR-Cas9 was utilized to knock out *FAD2* gene using two sgRNA. The knocked-out plants showed higher oleic acid content as compared to wild-type plants (Bahariah et al. 2021).

The demand for fragrant rice, particularly Indian Basmati, is gaining worldwide due to the presence of a characteristic fragrance in its grains. This fragrance is due to the presence of defective *OsBAD2* which favors the production of 2AP, one of the most abundant components of various volatile compounds responsible for fragrance (Zafar et al. 2020). Fragrance gene *BADH2* of Zhonghua 11 rice was edited using CRISPR/Cas9. An additional base (T) was introduced in the first exon of BADH2, leading to higher 2AP content in edited rice (Shao et al. 2017). CRISPR-Cas9 was applied to alter the *BADH2* gene in Zhengdao 19, a rice variety suited for direct sowing. The 2AP content in field planted $T_0$ was found to be increased from 0.003 μg/g (in the wild type) to 1.259 ± 0.072 μg/g for $T_0$ mutants. In greenhouse-planted $T_1$, it increased from 0.002 μg/g (in the wild type) to 0.537 ± 0.111 μg/g for $T_1$ mutants (Fuhua et al. 2018). A significant increase in grain yield and fragrance (2AP) was observed in CRISPR-edited rice. The mutants exhibited 2AP levels ranging from 0.72–0.78 mg/kg while 2AP was absent in wild type (Usman et al. 2020). CRISPR was used to introduce aroma in elite rice variety ASD16 by creating novel alleles of *OsBADH2* gene. SgRNA-mediated mutations were introduced in the seventh exon of *OsBADH2* gene. Novel aromatic comparatives, viz. pyrrolidine, pyridine, pyrazine, pyridazine, and pyrozole, were detected in the comparative volatile profiling of $T_1$ progenies grains (Ashokkumar et al. 2020).

Rice, one of the staple crops worldwide, is known to be deficient in vitamin A. Golden rice was developed to overcome this deficiency by enhancing the β-Carotene content in the endosperm (Paine et al. 2005). CRISPR-Cas has also been

utilized to improve the vitamin A content in rice. An ortholog of *Orange* (*Or*) gene in cauliflower, the *Osor* gene in rice was targeted using CRISPR-Cas9. Accumulation of orange color in the callus showed the enhanced β-carotene level in edited rice (Endo et al. 2019). A 5.2 kb carotenoid biosynthesis cassette at two genomic safe harbors was introduced in rice. An enhancedβ-carotene level was detected in seeds with no change in yield. Whole-genome sequencing revealed that no off-target mutations were created in Cas9 engineered plants (Dong et al. 2020).

Soft rice with 7–10% amylose content is quite popular in south China. The amylose content is determined by *Waxy* (*Wx*) gene. CRISPR-Cas9 has been recently applied to alter this gene to produce softer versions of elite cultivars. A loss of function in *Wx* gene of two widely cultivated elite japonica varieties, Xiushui134 (XS134) and Wuyunjing 7 (9522), was introduced by CRISPR-Cas9. A two-fold increase in gel consistency (GC) and a marked reduction in gelatinization temperature (GT) for CRISPR-waxy seeds were observed as compared to wild (Zhang et al. 2018a, b). The *Wx* gene of two elite rice cultivars, Huaidao 5 (HD5) and Suken 118 (SK118), was targeted to develop soft rice. The amylose content in the edited lines was around 2.6%–3.2% (Yunyan et al. 2019). Three elite rice cultivars, viz. Suijing 18 (SJ18), Songjing 2 (SJ2), and Longqingdao 3 (LQD3), were targeted for CRISPR-based editing of *Waxy* gene. The edited lines showed a significant reduction in the amylose content (Li et al. 2020a). An elite indica variety TianFengB was targeted to improve the cooking quality by reducing the amylose content using CRISPR-Cas9. The edited lines showed a significant reduction in amylose content. The study showed that in some of the edited lines the amylose content was similar to glutinous rice (Zeng et al. 2020). Cereal grains with higher starch content are known to be a good source of resistant starch (Jiang et al. 2010). Resistant starch is a form of nondigestible starch and is not absorbed in the body and protects from various noninfectious diseases (Regina et al. 2006). Keeping in mind the health benefits of resistant starch, CRISPR has been utilized to increase the starch content in rice. The calli derived from *japonica* cv. Kitaake was targeted for introduction of CRISPR-mediated mutagenesis in starch branching enzymes (SBE, SBEI, and SBEIIb). The *SBEI* and *SBEII* mutants showed a significant increase in amylose content and resistant starch by 25.0 and 9.8%, respectively (Sun et al. 2017). In another study, CRISPR-Cas9-mediated mutagenesis in *SBEII* resulted in increased amylose and resistant starch content from 19.6 to 27.4% and from 0.2 to 17.2%, respectively (Baysal et al. 2020).

Proanthocyanidins and anthocyanins are the major health-promoting nutrients present in rice. The red color in rice is governed by two recessive complementary genes, *Rc* and *Rd*. The wild species *Oryza rufipogon* has RcRd genotype which is responsible for red pericarp. However, a 14-bp frame-shift deletion in the seventh exon of *Rc* gene results in white phenotype in most of the cultivated rice varieties. Recently, this frame-shift mutation in recessive Rc was revered with the help of CRISPR-mediated editing, resulting in the conversion of white cultivar to red cultivar. The mutants showed high accumulation of proanthocyanidins and anthocyanins than the wild type. No significant difference in other agronomic traits was observed in the mutants as compared to wild type (Zhu et al. 2019).

## 14.14 Wheat

Wheat is an economically important cereal that supplies 20% of the calorie intake to over 60% of the global population. Presently, it is cultivated on around 220 million hectares with an annual production of 700–750 million tons and used in a wide range of products. The continued economic development has led to increased demand for premium quality wheat with improved grain quality. Grain quality is a mutagenic trait and the hexaploid genome of around 16 Gb with around 85% repetitive elements makes it more complex (Zhang et al. 2021). The advent of CRISPR-Cas9 has allowed researchers to create novel allelic variations to improve wheat grain quality. Recently, a web-based tool has been developed to design sgRNA for GE in wheat (Cram et al. 2019).

Celiac disease (CD) is the most common disease associated with wheat. It is an autoimmune disease prevalent in around 1–2% of the global population (Jouanin et al. 2020). Among food intolerances, CD is relatively well-understood from the standpoint of human immunity (Tye-Din et al. 2010). Gluten-free diets (GF), which exclude all wheat products, are the only way to prevent CD and this is very difficult as wheat gluten is present in almost every product. In the case of wheat, the gene family responsible for this autoimmune disease is α-gliadin, of which 33-mer is most immunogenic. The coding region of 33-mer was targeted using CRISPR and 21 mutant lines were developed. A total of 35 different genes were mutated and the immunoreactivity was reduced by 85%. These GE lines could be utilized in making low-gluten foodstuffs (Sánchez-León et al. 2018). In another study, both α- and γ-gliadins were targeted using CRISPR-Cas9. The $T_1$ generation showed altered gliadins production (Jouanin et al. 2019). Two genes related to grain quality, *pinb* (grain hardness) and *waxy* (starch quality), and one for kernel size *DA1* were mutated using CRISPR. Three mutant lines were generated and a mutation efficiency of 54.17% was recorded (Zhang et al. 2018a, b). Two subunits of α-amylase/trypsin inhibitors (ATI), viz. WTAI-CM3 and WTAI-CM16, were targeted to reduce allergen proteins in durum wheat using CRISPR (Camerlengo et al. 2020). The resistant starch content of modern wheat varieties is quite low. Targeted mutagenesis of *TaSBEIIa* using CRISPR was employed to increase amylose content in winter wheat *cv.* Zhegmai 7698 (ZM) and spring wheat *cv.* Bobwhite. Flour quality analysis showed that the triple-null lines possessed significantly increased amylose content (Li et al. 2020b). Four-grain quality genes, viz. *pinb* (grain hardness), *waxy* (starch quality), *ppo,* and *psy* (dough color), were targeted using CRISPR-Cas9. The mutants showed a significant reduction in expression of all four genes (Zhang et al. 2021).

## 14.15 Barley (*Hordeum Vulgare*)

Barley is one of the first crops to be domesticated, a major constituent of the brewing industry and primarily used as feed and food. Like other cereals, the presence of phytic acid is a major drawback associated with barley (Cosgrove 1980).

CRISPR-Cas9-mediated mutations in the promoter of the barley phytase gene *HvPAPhy_a* showed reduced mature grain phytase activity (Holme et al. 2017). In another study, *HvITPK1* gene responsible for phytic acid production in barley was mutated. The mutants contained altered levels of phosphate in the mature grains, ranging from 65% to 174% of the wild-type content (Vlčko and Ohnoutková 2020). D-hordein component of barley storage protein was mutated using CRISPR. Barley grains without D-hordein protein in $T_2$ seeds showed a decrease in the prolamines and an increase in the glutenins. Further, there was increase in starch, amylose, and β-glucan content (Yang et al. 2020).

## 14.16 Sorghum (*Sorghum Bicolor*)

Sorghum, a drought-tolerant crop, is a major staple food and feed crop in semiarid regions where cultivation of other cereals is not possible. Sorghum is deficient in essential amino acids like lysine and its protein is difficult to digest (Aboubacar et al. 2001). The reason behind the nondigestibility is the presence of prolamins, known as kafirins, which account for 70% of the total seed protein (Hamaker et al. 1995). Kafirins mainly comprise α-kafirins encoded by gene family *k1C*. The gene family *k1C* was targeted using CRISPR. A reduced T1 and T2 α-kafirin and increased grain protein digestibility and lysine content were observed in $T_2$ generation seeds (Li et al. 2018).

## 14.17 Conclusion and Future Prospects

Over the past several decades, conventional and molecular breeding has made significant contributions to agriculture. They utilize the variations in the available germplasm to develop improved versions. However, the improvement through these technologies is limited due to nonavailability of diverse germplasm. Moreover, these methods are time- and labor-intensive. GE, an advanced biotechnological tool, can be utilized to overcome the limitations. GE opens new avenues for researchers in the field of plant sciences to modify the target trait to interest, leading to a prodigious success in plant biotechnology. The use of genome editing has allowed efficient, rapid, specific, and targeted editing for increasing yield and nutritional value in cereals and other horticultural and fruit crops. These technologies add an edge to the existing genetic engineering struggles in attaining food security and combating malnutrition with the increasing global population. As GE crops are not likely to undergo strict regulations as in the case of GM crops (Yadav et al. 2018), they have come up as the first choice of researchers to develop improved crop plants. Despite several advancements in the field of GE, several challenges are associated with GE in cereals. Some of the major challenges are development of efficient transformation protocol, off-target effects, multiplexing, requirement of specific promoters,

and complex design (Ansari et al. 2020). There are also certain restrictions and regulations for application of GE to crops (Zhang et al. 2020). All these challenges must be resolved to utilize GE to its full potential. The development of crop plants with enhanced nutritional value using GE promises that everyone gets a healthier diet.

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