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Advance breeding and biotechnological approaches for crop improvement: A review

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Abstract
In this changing climate scenario, rapid increase of human population resulted in increased demand of food production. During the last century crop plants have been improved through classical breeding techniques and numerous varieties of several crops have been developed across the world. However conventional breeding in improving crop plants is constrained due to genetic erosion, genetic drag, reproductive obstacles and usually take longer time. Thus, there is an urgent need for the novel breeding and biotechnology-assisted crop improvement, which ultimately aimed to obtain novel plant traits. Many novel techniques such as marker assisted selection, marker assisted back cross breeding, marker assisted gene pyramiding plays crucial role in improvement of crop plants. Advancement in plant genetic engineering (genetic transformation and genome editing) have made it possible to transfer gene into crop plants from unrelated plants and even from non-plant organism. These biotechnological approaches are a great option to improve crop plants with significant commercial properties such as increased biotic stress resistant or abiotic stress tolerances; nutrition; yield and quality.

Keywords: marker, breeding, biotechnology, improvement, genome

1. Introduction
In this changing climate scenario, rapid increase of human population resulted in increased demand of food production. The current world population of 7.3 billion is expected to reach 9.7 billion in 2050. “FAO estimates that we have to double food production by 2050 to feed the expected 9 billion people, knowing that one billion people are already going to bed hungry every day." (Rijsberman 2012) [34]. Plant breeding will play very important role in this coordinated effort for increased food production. Given the context of current yield trends, predicted population growth and pressure on the environment, traits relating to yield stability and sustainability should be a major focus of plant breeding efforts. These traits include durable disease resistance, abiotic stress tolerance and nutrient and water-use efficiency (Mackill *et al*. 1999 and Trethowan *et al*. 2005) [20, 43]. Despite optimism about continued yield improvement from conventional breeding, new technologies such as biotechnology will be needed to maximize the probability of success (Huang *et al*. 2002) [10]. Development of molecular (DNA) markers has created a powerful and practicable tool to perform gene selection in plant breeding, although it is not a real gene selection but the best indirect selection for target genes at the DNA level. By using DNA markers to assist in plant breeding, efficiency and precision could be greatly increased. The use of DNA markers in plant breeding is called marker-assisted selection (MAS). Many novel techniques such as marker assisted selection, marker assisted back cross breeding, marker assisted gene pyramiding plays crucial role in improvement of crop plants. Advancement in plant genetic engineering (genetic transformation and genome editing) have made it possible to transfer gene into crop plants from unrelated plants and even from non-plant organism. These biotechnological approaches are a great option to improve crop plants with significant commercial properties such as increased biotic stress resistant or abiotic stress tolerances; nutrition; yield and quality.

2. Molecular marker -assisted breeding or Molecular-assisted breeding
Molecular-assisted breeding (MAB), is the use of molecular technologies (particularly DNA markers) with linkage maps and genomics approaches, to edit and improve trait of interest on the basis of genotypic analyses (Jiang 2013) [11].

Molecular-assisted breeding describes various novel breeding approaches, comprising marker-assisted selection (MAS), marker-assisted backcrossing (MABC), marker-assisted recurrent selection (MARS), and genomic selection (GS) (Ribaut 2010) [32]. This is considered as a novel approach and a potential methodology to improve genetic make-up of plants. Moreover, it has been applied extensively in many crop plants including horticultural crops.

2.1 Marker-assisted selection (MAS)

Marker-assisted selection or marker aided selection (MAS) is an indirect method of selection whereby a phenotype is selected based on a marker (morphological, biochemical or DNA/RNA variation) associated to a trait of interest (e.g. productivity, disease resistance, abiotic stress tolerance, and quality), instead of the trait itself the genotype of a marker (Rosyara 2006) [35].

2.2 Marker-assisted or marker-based backcrossing (MABC)

Marker -assisted backcrossing (MABC) is considered as the easiest form of marker-assisted selection, intends to transfer targeted genes/QTLs from a genetic source (donor parent) into a superior variety or elite breeding line (recurrent parent) to improve the trait of interest and the backcrossing totally based on the markers lined to gene or QTL of interest rather than phenotypic performance of trait of interest. Moreover, It is an important and advantageous approach when phenotyping is difficult, heritability of trait of interest is low, the expression of trait in later stages of growth and development of plants (such as flowers, fruits, and seeds), the traits are governed by genes that need a particular condition to express, the traits are governed by recessive genes, and gene pyramiding is required for the traits (Jiang 2013) [11]. It has been practised in various kind of traits i.e. disease and pest resistance, drought tolerance and quality related traits in many crop species like rice, wheat, maize, barley, pear millet, soybean, tomato, etc. (Collard *et al*. 2005 and Dwivedi *et al*. 2007) [6, 8].

2.3 Marker -assisted gene pyramiding

Marker-assisted gene pyramiding has been aimed and used to improve resistance against diseases and pests by targeting two or more genes simultaneously (Jiang 2013) [11]. For instance, gene pyramids has been developed in paddy against bacterial blight and blast (Singh *et al*. 2011) (Luo and Yin 2013) [19]. Similarly, in barley successful pyramiding of qualitative gene and QTLs for resistance to stripe rust also reported (Castro *et al*. 2003) [3]. In this case molecular markers which linked to genes or OTLs of interest has great importance and allows precise selection of targeted traits at initial stage of plant growth and developments. Furthermore, it is a potential approach to improve quantitatively inherited characters in plants and pyramiding of multiple genes or QTLs (Richardson *et al*. 2006) [33]. The pyramiding of multiple QTL and their cumulative effect has been reported in many crop plants such as wheat, barley, and soybean (Li *et al*. 2010 and Wang *et al*. 2015) [17, 37].

2.4 Marker-assisted recurrent selection (MARS)

Pyramiding of genes or QTLs with the help of molecular markers is more cumbersome and less practised while, the recurrent selection is considered as competent approach for the improvement of quantitative traits. Moreover, the selection's effectivity and efficiency are unsatisfactory some times because phenotypic selection is purely based on environments whereas, the genotypic selection is a tedious and time-consuming job (One selection cycle need at least 2-3 cropping season). Marker -assisted recurrent selection is a strategy which appropriates genotypic selection and intermating in the same cropping season for one cycle of selection. Consequently, it could enhance the efficiency of recurrent selection and accelerate the progress of the procedure**.** It is especially useful in introgression of multiple targeted genes or QTLs from various sources through recurrent selection based on a multiple-parental population [18].

2.5 Genomic selection (GS) or genome -wide selection (GWS)

Genomic selection is a kind of marker-based selection, concerning to the simultaneous selection for a large numbers of markers, which deals the complete genome in a dense way so that all genes are assumed to be in linkage disequilibrium with at least few of the markers (Meuwissen 2007) [23]. In this method genotypic data of the entire genome are applied to predict complicated traits precisely to grant the selection on the prediction only. Selection of desirable plants is totally based on genomic estimated breeding value (GEBV) (Nakaya and Isobe 2012) [25]. GEBV is a predicted breeding value estimated by using genome wide dense DNA markers**.** In addition to that genomic selection can avoid the requirement to search the significant QTL-marker loci correlations individually (Desta and Ortiz 2014). In the other terms, in the genomic selection, QTL mapping with populations which obtained from particular crosses can be skipped whereas it is a need to formulate GS models which is the formulae for genomic estimated breeding value prediction. In this statistical approaches are first used to predict significant correlation between genotypes and phenotypes by the investigation of phenotypes and genotypes in the training populations (a subset of a population). Afterward, genomic estimated breeding values are in the action to select desirable individuals in the breeding process, rather than the genotypes of markers practised in marker-assisted selection (Jiang 2013) [11].

3. Genetic Transformation

Plant genetic engineering has opened new avenues to modify crops, and provided new solutions to solve specific needs (Rao *et al*. 2009) [31]. Contrary to conventional plant breeding, this technology can integrate foreign DNA into different plant cells to produce transgenic plants with new desirable traits (Newell 2000) [27]. These biotechnological approaches are a great option to improve crop genotypes with significant commercial properties such as increased biotic (resistance to disease of virus, fungi, pests and bacteria) (Fagoaga *et al*. 2007) [9] or abiotic (temperature, salinity, light, drought) stress tolerances; nutrition; yield and quality (delayed fruit ripening and longer shelf life) and to use as bioreactor to produce proteins, edible vaccines and biodegradable plastics (Khandelwal *et al*. 2011) [4]. Currently, public concerns and reduced market acceptance of transgenic crops have promoted the development of alternative marker free system technology as a research priority, to avoid the use of genes without any purpose after the transformation protocol as selectable and reporter marker genes. Typically, it is employed for the selection strategy that confers resistance to antibiotics and to herbicides.

3.1 Transgenic

Genetically engineered organisms are referred as transgenic. In other words, a genotype developed by the process of genetic engineering is called transgenic. It may be a plant, an animal or microbes such as fungi, bacteria and viruses. Transgenic plants are obtained involving tissue culture and genetic engineering techniques.

3.2 Cisgenics: Other relevant advance in genetic transformation was the proposal made by (Schouten *et al*. 2006), the "cisgenesis". This term means the use of recombinant DNA technology to introduce genes from crossable donors plants, isolated from within the existing genome or sexually compatible relative species for centuries therefore, unlikely to alter the gene pool of the recipient species. Cisgenesis includes all the genetic events of the T-DNA as introns, flanking regions, promoters, and terminators. Cisgenesis is one of the new plant breeding technologies emerging as a promising tool for the future, more publicly accepted than the traditional transgenic approach.

4. Genome editing

The most forward-looking crop genome modification technology is genome editing. Genome editing is a mighty technology developed for precisely and site-specifically addition, modification or deletion of gene of interest from genome. Genome editing is utilized in improvement of basic understanding of gene functions of plants. Genome editing comprises ZFNs (zinc-finger nucleases), TALENs (transcription activator-like effector nucleases), and CRISPR/Cas (clustered regularly interspersed short palindromic repeats) systems. It is also applied for the improvement in important traits of crop plants. For example, ZFN system was exploited for the precisely and site-specifically insertion of a transgene expression cassette in order to develop the herbicide tolerance corn (Shukla *et al*. 2009). In spite of that, aromatic rice developed via TALEN (Shan *et al*. 2015) [28] a powdery mildew resistant wheat also developed via the CRISPR/Cas system (Shan *et al*. 2014) [27]. Though the these genome editing technologies can be used for elimination of the undesirable genetic sequences from plant genomes, a most recent genome editing system called "piggy Bac" might be the best choice (Nishizawa *et al*. 2015) [28] for excising of any undesirable sequences of DNA. In spite of everything, the genome editing systems are used, the crop that is genome edited is called "subgeneic" (Wang *et al.* 2014) [37].

4.1 Zinc Finger Nucleases (ZFNs)

The science of Zinc Finger Nucleases was began in Johns Hopkins University when the scientist were trying to construct a new restriction enzyme (Kim *et al*. 1996) [15]. They were working on *Fok*I (a type IIS restriction enzymes) which distinguish specific sequences of DNA and cleave thousands of base pair 5–3 downstream of the recognition site. The aim of the study was to fuse the *Fok*I catalytic domain to a protein domain which recognises and binds to DNA to change restriction enzyme specificity. Subsequently, the scientist selected zinc finger (ZF) proteins as the domain of protein to recognize DNA. Zinc finger (ZF) proteins generally recognise three nitrogenous bases blocks in single sequential order. The pioneer report of ZFNs in plants were first studied in model plants like *Arabidopsis thaliana* and *Nicotiana tabacum* by (Lloyd *et al*. 2005 and Wright *et al*. 2005) respectively.

4.2 Transcription Activator-like Effector Nucleases (TALENs)

The *Xanthomonas* is a Phyto Phathogenic bacteria, causes infection in many crop plants comprising rice, citrus, tomato, cole crops, and soybean**.** At the time of infection, the bacteria inoculated in vegetative cells and produce a chain of proteins which is known as transcription activator-like effectors (TALEs) (Boch and Bonas 2010) [1] (Bogdanove *et al*. 2010) [2] These TALEs proteins binds to a particular promoter's sequences of hosts and imitating transcription factors of them (Kay and Bonas 2009) [29]. Moreover, TALEs contains a DNA binding domain which generally having 16-20 monomeric repeats. The each monomer is highly conserved and contains total 34 amino acids. However, the hypervariable amino acid residues at 12 and 13 positions are the exception, which are called as repeat-variable di-residues (RVDs). Currently, molecular biology and bioinformatics analysis have allowed the decoding of the TALE code to recognise DNA (Moscou and Bogdanove 2009) [24] Each of the RVD recognises a specific DNA base; for instance, NI repeats of RVDs bind to adenosine, HD to cytosine, NG to thymine, and NN to guanine or adenosine (Nakayama *et al*. 2014). After the elucidation of DNA recognition mechanism by TALEs got attention instantly for its utilisation in biotechnology (Bogdanove *et al*. 2010) [2] In foremost experiment, The TALE binding domain was fuse to the *Fok*I endonuclease catalytic domain, which making TALENs. The coalition of binding domain for personalised or native DNA sequences with *Fok*I caused specific double-stranded DNA break (DSB) production (Christian *et al* 2011) [4]. It have been applied to get site-specific modifications in various plant species like *A. thaliana* (Cermak *et al*. 2011) [4], tobacco (Mahfouz *et al*. 2012) [21], and rice to developed heritable, disease-resistant lines.

4.3 Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR/Cas9)

The newly discovered CRISPR-Cas9 nuclease is guided by short guide RNA that pair via Watson-Crick base pairing with its target DNA sequence and it overcomes the challenges of previously used nucleases. The ease of cas-9 targeting, efficient site specificity and ability for multiple editing made the CRISPR-Cas9 system more popular and helped in opening a wide range of practical applications in the medical and crop improvement research field (Song *et al*. 2016 and Paul *et al*. 2016) [42, 30]. Nowadays CRISPR is being used not only for genome editing purposes but also for other gene expression regulation and epigenetic modifications. After completion of genome editing in the plant transgene (Cas9 or SgRNA) free plants can be obtained in next few segregating generations. This overcomes the traditional limitations of genetically modified crops which suffers much because of the presence of transgenes (Patidar *et al*. 2016) [29].

5. Conclusion

The breeding or improvement of plants has begun with the domestication of the crop plants. The modern breeding of crops totally based on the basic principles of inheritance and it has become the most prominent element of world agriculture. In addition, the traditional breeding approaches are utilising successfully in the varietal improvement and germplasm conservation of the crops. However, the traditional breeding is still based on the rigorous evaluation and selection process.

Molecular marker-assisted breeding provides great opportunities for plant breeder to evaluate germplasm, map genes, and characterise complex traits effortlessly. Moreover, it permits selection at seedling stage which reduced the breeding cycle of the crop and so we can able to breed a variety in shorter time period. It also offers to perform selection in all the climatic conditions e.g. greenhouse and off-season nurseries. The genotyping of plants by using molecular markers is quicker, cheaper and precise than the traditional phenotyping. Similarly, the trending technology is genome editing which is a mighty technology developed for precisely and site-specifically addition, modification or deletion of gene of interest from genome. These technologies are highly effective and efficient in terms of resources, effort and time and having great potential to combat and solve the high population and hunger questions by the developments of crop varieties having higher yield and nutrients potential with resistant to diseases and pests.

6. References

1. Boch J, Bonas U. Xanthomonas AvrBs3 family-type III effectors: discovery and function. Annual review of phytopathology, 2010, 48.
2. Bogdanove AJ, Schornack S, Lahaye T. TAL effectors: finding plant genes for disease and defense. Current opinion in plant biology. 2010; 13(4):394-401.
3. Castro AJ, Capettini F, Corey AE, Filichkina T, Hayes PM, Kleinhofs A *et al*. Mapping and pyramiding of qualitative and quantitative resistance to stripe rust in barley. Theoretical and Applied Genetics. 2003; 107(5):922-930.
4. Cermak T, Doyle EL, Christian M, Wang L, Zhang Y, Schmidt C *et al*. Efficient design and assembly of custom Talen and other TAL effector-based constructs for DNA targeting. Nucleic acids research. 2011; 39(12):82-82.
5. Christian M, Tomas C, Erin LD, Clarice S, Feng Z, Aaron H et al. Targeting DNA double-strand breaks with TAL effector nucleases. Genetics. 2010; 186(2):757-761.
6. Collard BCY, Jahufer MZZ, Brouwer JB, Pang ECK. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. Euphytica. 2005; 142(1-2):169-196.
7. Desta ZA, Ortiz R. Genomic selection: genome-wide prediction in plant improvement. Trends in plant science. 2014; 19(9):592-601.
8. Dwivedi SL, Crouch JH, Mackill DJ, Xu Y, Blair MW, Ragot M *et al*. The molecularization of public sector crop breeding: progress, problems, and prospects. Advances in agronomy. 2007; 95:163-318.
9. Fagoaga C, Tadeo FR, Iglesias DJ, Huerta L, Lliso I, Vidal AM *et al*. Engineering of gibberellin levels in citrus by sense and antisense overexpression of a GA 20-oxidase gene modifies plant architecture. Journal of Experimental Botany. 2007; 58(6):1407-1420.
10. Huang JK, Pray C, Rozelle S. Enhancing the crops to feed the poor. Nature. 2002; 418:678-684. (doi:10.1038/ nature01015)
11. Jiang GL. Plant marker-assisted breeding and conventional breeding: challenges and perspectives. Adv. Crop Sci. Technol. 2013; 1:e106.
12. Jiang GL, Dong Y, Shi J, Ward RW. QTL analysis of resistance to Fusarium head blight in the novel wheat germplasm CJ 9306. II. Resistance to deoxynivalenol accumulation and grain yield loss. Theoretical and Applied Genetics. 2007; 115(8):1043-1052.
13. Kay S, Bonas U. How Xanthomonas type III effectors manipulate the host plant. Current opinion in microbiology. 2009; 12(1):37-43.
14. Khandelwal A, Renukaradhya GJ, Rajasekhar M, Sita GL, Shaila MS. Immune responses to hemagglutinin-neuraminidase protein of Peste des petits ruminants virus expressed in transgenic peanut plants in sheep. Veterinary Immunology and Immunopathology. 2011; 140:291-296.
15. Kim YG, Cha J, Chandrasegaran S. Hybrid restriction enzymes: zinc finger fusions to Fok I cleavage domain. Proceedings of the National Academy of Sciences. 1996; 93(3):1156-1160.
16. Li T, Liu B, Spalding MH, Weeks DP, Yang B. High-efficiency TALEN- based gene editing produces disease-resistant rice. Nature biotechnology. 2012; 30(5):390-392.
17. Li X, Han Y, Teng W, Zhang S, Yu K, Poysa V *et al*. Pyramided QTL underlying tolerance to Phytophthora root rot in mega-environments from soybean cultivars 'Conrad' and 'Hefeng 25'. Theoretical and applied genetics. 2010; 121(4), pp.651-658.
18. Lloyd A, Plaisier CL, Carroll D, Drews GN. Targeted mutagenesis using zinc-finger nucleases in Arabidopsis. Proceedings of the National Academy of Sciences of the United States of America. 2005; 102(6):2232-2237.
19. Luo Y, Yin Z. Marker-assisted breeding of Thai fragrance rice for semi-dwarf phenotype, submergence tolerance and disease resistance to rice blast and bacterial blight. Molecular breeding. 2013; 32(3):709-721.
20. Mackill DJ, Nguyen HT, Zhang J. Use of molecular markers in plant improvement programs for rainfed lowland rice. Field Crops Res. 1999; 64:177-185. (doi:10.1016/S0378-4290(99)00058-1).
21. Mahfouz MM, Li L, Piatek M, Fang X, Mansour H, Bangarusamy DK *et al*. Targeted transcriptional repression using a chimeric TALE-SRDX repressor protein. Plant molecular biology. 2012; 78(3):311-321.
22. Manimaran P, Ramkumar G, Sakthivel K, Sundaram RM, Madhav MS, Balachandran SM. Suitability of non-lethal marker and marker-free systems for development of transgenic crop plants: Present status and future prospects. Biotechnology Advances. 2011; 29:703-714.
23. Meuwissen T. Genomic selection: marker assisted selection on a genome wide scale. Journal of animal Breeding and genetics. 2007; 124(6):321-322.
24. Moscou MJ, Bogdanove AJ. A simple cipher governs DNA recognition by TAL effectors. Science. 2009; 326(5959):1501-1501.
25. Nakaya A, Isobe SN. Will genomic selection be a practical method for plant Breeding. Annals of botany. 2012; 110(6):1303-1316.
26. Nakayama TJ, Borém A, Chiari L, Molinari HBC and Nepomuceno AL. Precision Genetic Engineering. Omics in Plant Breeding, 2014, 187-205.
27. Newell CA. Plant transformation technology; developments and applications. Molecular Biotechnology. 2000; 16:53-65.
28. Nishizawa-Yokoi A, Endo M, Ohtsuki N, Saika H, Toki S. Precision genome editing in plants via gene targeting and piggy Bac-mediated marker excision. The Plant Journal. 2015; 81(1):160-168.

29. Patidar OP, Gautam C, Tantuway G, Kumar S, Yadav A, Meena DS, Nagar A. RNA-guided Genome Editing Tool CRISPR-Cas9: Its Applications and Achievements in Model and Crop Plants. Journal of Pure and Applied Microbiology. 2016; 10(4):3035-3042.
30. Paul JW, Qi Y. CRISPR/Cas9 for plant genome editing: accomplishments, problems and prospects. Plant cell reports. 2016; 35:1417.
31. Rao AQ, Bakhsh A, Kiani S, Shahzad K, Shahid AA, Husnain T *et al*. The myth of plant transformation. Biotechnology Advances. 2009; 27:753-763.
32. Ribaut JM, De Vicente MC, Delannay X. Molecular breeding in developing countries: challenges and perspectives. Current Opinion in Plant Biology. 2010; 13(2):213-218.
33. Richardson KL, Vales MI, Kling JG, Mundt CC, Hayes PM. Pyramiding and dissecting disease resistance QTL to barley stripe rust. Theoretical and Applied Genetics. 2006; 113(3):485-495.
34. Rijsberman F. CGIAR: a global research partnership for a food secure future. Retrieved, 2012, 2/5/13, from http://www.cgiar.org/consortiumnews/cgiar-global-research-partnership-for-a-food-secure-future/
35. Rosyara UR. Requirement of robust molecular marker technology for plant breeding applications. Journal of Plant Breeding Group. 2006; 1:67-72.
36. Schouten HJ, Krens FA, Jacobsen E. Do cisgenic plants warrant less stringent oversight? Nature Biotechnology. 2006; 24:753.
37. Shan Q, Wang Y, Li J, Gao C. Genome editing in rice and wheat using the CRISPR/Cas system. Nature protocols. 2014; 9(10):2395-2410.
38. Shan Q, Zhang Y, Chen K, Zhang K, Gao C. Creation of fragrant rice by targeted knockout of the OsBADH2 gene using TALEN technology. Plant biotechnology journal. 2015; 13(6):791-800.
39. Shukla VK, Doyon Y, Miller JC, DeKelver RC, Moehle EA, Worden SE *et al*. Precise genome modification in the crop species Zea mays using zinc-finger nucleases. Nature. 2009; 459(7245):437-441.
40. Singh S, Sidhu JS, Huang N, Vikal Y, Li Z, Brar DS *et al*. Pyramiding three bacterial blight resistance genes (xa5, xa13 and Xa21) using marker-assisted selection into indica rice cultivar PR106. Theoretical and Applied Genetics. 2001; 102(6-7):1011-1015.
41. Slafer GA, Araus JL, Royo C, Del Moral LFG. Promising eco-physiological traits for genetic improvement of cereal yields in Mediterranean environments. Ann. Appl. Biol. 2005; 146:61-70. (doi:10.1111/j.1744-7348.2005.04048.x).
42. Song G, Jia M, Chen K, Kong X, Khattak K, Xie C *et al*. CRISPR/Cas9: A powerful tool for crop genome editing. The crop journal. 2016; 4(2):75-82.
43. Trethowan RM, Reynolds M, Sayre K, Ortiz Monasterio I. Adapting wheat cultivars to resource conserving farming practices and human nutritional needs.Ann. Appl. Biol. 2005; 146:405-413. (doi:10.1111/j.1744-7348.2005.040137.x).
44. Wang X, Jiang GL, Song Q, Cregan PB, Scott RA, Zhang J *et al*. Quantitative trait locus analysis of seed sulfur-containing amino acids in two recombinant inbred line populations of soybean. Euphytica. 2015; 201(2):293-305.
45. Wang Y, Cheng X, Shan Q, Zhang Y, Liu J, Gao C *et al*. Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Nature biotechnology. 2014; 32(9):947-951.
46. Wright DA, Townsend JA, Winfrey RJ, Irwin PA, Rajagopal J, Lonosky PM *et al*. High-frequency homologous recombination in plants mediated by zinc-finger nucleases. The Plant Journal. 2005; 44(4):693-705.
47. Xu Y. Molecular plant breeding. CAB International, Wallingford, UK/Cambridge, MA, 2010.