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Potential of Molecular Plant Breeding for Sustaining the Global Food Security

Ritu Mahajan* **and Nisha Kapoor**

School of Biotechnology, University of Jammu, Jammu and Kashmir, India.

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New alleles are continuously added to bring novel traits which are selected using genetic manipulations. Understanding the genes responsible for a particular phenotype involves recent genomic approaches which are to be integrated with conventional breeding programs for the crop improvement. Use of biotechnological tools merged with the conventional breeding practices has resulted molecular plant breeding which has significant contributions to food production, despite the presence of narrow genetic base in current materials used for breeding purposes. Use of molecular markers along with high-throughput genome sequencing efforts, have dramatically augmented our information to characterize the elite germplasm for the production of hybrids and improved populations. This review focuses on possibilities for the need and application of molecular breeding tools in the genetic improvement of the crop plants which can ensure sustainable food production for the increasing global population.

Keywords: Genetic Diversity; Marker Assisted Selection; Molecular Markers; Plant Breeding, Sequencing.

Agriculture being dominant in the world today, hunger and malnutrition continue even in developing countries. With the increase in the global population, the rate of food production needs to be doubled, so as to feed ten billion people by the year 2050 [1]. Although, the growth rate of the crop yield is 2.4% per annum but still the yield of major food crops or cash crops is low [2], and still the increase in crop production recorded is far less than half the required rate. Over a century, a remarkable progress has been made in plant breeding with the mandate to increase the crop yield and this was made possible due to the huge developments in the fields of agricultural technology. Since, most of the traits in breeding programs are quantitative in nature so the selection in classical breeding is based on phenotype rather than genotype and this result in the selection of favorable genes in the course of time [3]. Plant breeding has the ability to alter the traits of plants where the desired characteristics related to specific traits are selected and this results in the production of certain elite varieties with superior agricultural characters.

To feed the world population, enhancement by 70% in the current food production is required, but mostly due to unfavorable consequences of climatic changes and variations, the crop yield is badly affected thus causing a threat to food security [4]. Also, with time, the agricultural land and water resources are becoming stagnant and thus that the desired increase in crop production can be attained by eco-efficient crop production systems.

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*Corresponding author E-mail: ritufeb@gmail.com

However, the major challenge still lies in doubling the food production but at the same time without compromising with environmental integrity and public health. Thus, plant breeding needs re-orientation so as to generate 'smart' crop varieties that have higher yield with fewer inputs. Such crop plants can only be raised by the application of advanced molecular tools [5,6]. This will involve the re-engineering of plants with desirable traits and genetic stability along with the increase in food productivity by two to three folds.

In this review, we have discussed few molecular based methods that could be integrated into traditional plant breeding practices (Fig 1) to meet the food productivity challenges. Molecular based tools and marker-assisted selection has resulted in decrease in the time required for developing a new crop variety with desirable traits. Several earlier reviews have demonstrated the use of molecular markers in crop improvement but their reliability/reproducibility as selection criteria for various plant populations was still not clear. The present review focuses on the recent advancements made in the field of molecular biology and how they are used for enhancing the crop productivity either by introducing the new genes/alleles or by editing the genome therby opening new opportunities for basic research in the fields of plant biology (Table 2).

Forward and reverse mutagenesis

Plant variations occur due to mutations and crossings and in their absence, conventional breeding based on phenotypic selection would be not possible in the nature. Mutations are the primary source of genetic variations that provides the raw material for selection and is also a powerful force playing significant role in evolution [7]. Spontaneous mutations are uncommon with low mutation rate and since they occur naturally so it's difficult to utilize them in the plant breeding programs. Hence, certain constraints that occur in conventional breeding like low vigour and fertility, complex genomes, narrow gene pool, lengthy breeding cycle can be addressed through induced mutagenesis. Apart from them, transitions and transversions induce variations in base pair changes while additions and deletions results in alteration in reading frame while duplications change the gene sequence and its functioning. Thus, development of new varieties through mutation breeding along with recombination has added new traits to the elite germplasm.

The main aim for mutation-based breeding is to increase the number of new varieties or to improve already adapted plant varieties, which is generally done by modifying one or two major traits to increase the productivity or quality of plants [8]. Plant mutagenesis has introduced variations in barley crop plants where ethylmethanesulfonate (EMS) based mutated plant populations were screened for two genes followed by PCR-based mutation scanning and detection of dye labeled cleaved products [9].

Mutation-based breeding suggests a unique opportunity to recognize the novel traits in the population but at the same time, for complex traits, these variations could not be screened using simple genotypic or phenotypic screening methods. Thus, muta-genomics is becoming an essential tool where mutant traits are investigated using high throughput technologies for analyzing gene functions [10]. Several mutagenesis methods like transposon tagging along with T-DNA insertion and ionizing radiation have offered an important knowledge about the type of mutations [11]. Insertional mutagenesis or transposons results mainly in disordering the gene sequence as compared to EMS based chemical mutagenesis that results in point mutations [12].

Introducing chemical based mutations in the development of TILLING (Targeting Induced Local Lesions in Genomes) technology has resulted in the introduction of random mutations across the entire genome. TILLING involves extraction of genomic DNA from each mutated line and screening of the plant population using advanced molecular techniques [13]. TILLING technique has been applied successfully to barley, rice, wheat, maize, oat, pea and soybean [14,15]. Chawade et al.[16] developed a TILLING population of spring oat, which has a mutation frequency of one mutation per 20-40 kb and several mutations in some important genes that could develop oat resources with specific traits. Plant mutant repositories are being established as in barley HorTILLUS (Hordeum—TILLING—University of Silesia) platform, which is the largest and an eternal source for new mutations for barley researchers where a mutagenized population was created for spring barley cultivar with majority of transitions and few for transversions [17]. Thus, this technology is a combination of both high frequency of induced mutations and receptive techniques for discovering point mutations in the plant populations [18].

Germplasm variation and genetic diversity

The foundation for molecular plant breeding was shaped by the use of genetic manipulations at DNA level so as to improve plants with desired characters. This involves the integration of advanced biotechnological techniques along with the molecular marker applications. Without the introduction of DNA markers, the idea of discovering the loci controlling complex traits was impossible. Currently, using molecular markers these genome regions can be easily flagged, thereby allowing the selection for Quantitative trait loci (QTLs) (Table 1).

Increase in population pressure and expansion in agricultural land has resulted in the erosion of plant biodiversity which ultimately lead to the large-scale extinction of agriculturally important crop species. After pandemics such as the famous Irish potato famine and Southern corn leaf blight epidemic in USA, crop varieties with higher yields were introduced under green revolution to overcome the food crisis. As a result, many gene banks were initiated by the Consultative Group of International Agricultural Researches (CIGAR) and few centers for exploring research in conserving the genetic resources for various food crops around the world.

Genetic diversity is the foundation to biodiversity but inbreeding among plants has resulted in recombination between undesirable genes [19, 20]. Use of molecular tools in plant breeding resulted in expansion of genetic diversity and also provides opportunity to farmers and breeders in developing new varieties with improved characteristics. Though, the phenotypic variation has positive impact on genetic diversity, yet it is dependent upon the interactions between genotype and environmental factors [21]. This is due to the presence of variations in the phenotype which are maintained further in the successive generations and sometimes results in the origin of new plant species thus contributing to the genetic diversity [22].

Since, the genetic diversity changes over time under artificial selection hence more efforts are required to explore the crop genetic diversity so as to attain sustainable crop production in near future [23]. Domestication, natural selection and mutations have great impact on genetic diversity. Domestication reduces, while mutations increase the genetic diversity. Similarly, directional selection decreases while at the same time disruptive selection increases the genetic diversity [24].

Recent molecular techniques such as New Generation Sequencing, has gained importance due to its low cost, less time for sequencing the genome and the accumulation of huge data. Several software programs are accessible for estimating diversity at phenotypic and molecular level thus increasing the effectiveness of germplasm for crop improvement.

MAS for quantitative trait selection

Most of the traits in breeding programs are quantitative in nature; while in classical breeding selections are made on the basis of phenotype without taking into account the genes that gets selected in each consecutive generation [3]. Hence, enhancement in the crop yield under conditions such as water scarcity, degradation of arable land, pollution, biotic and abiotic stresses along with consumer preferences, has become a challenge for plant breeders and scientists to develop new crop varieties persistently.

With the arrival of molecular markers the likelihood of selecting the desirable traits has became easier and are used frequently in the plant breeding programs.. Marker-assisted selection (MAS), where the molecular markers are closely linked to gene of interest, has now a days become a feasible tool to select a particular trait in the breeding programs for several crop species [25,26,27]. Using associated markers, it is interpreted that MAS depends upon the genotype of plants rather than its phenotype· so it is an indirect selection of crops with improved traits based on the closely associated markers [28, 29], and once the association between marker and trait is linked then the desirable traits can be transferred from parents to offsprings through marker assisted backcrossing [30].

The main challenge faced by breeders is where introgression and multiple genes pyramiding (for polygenic traits) affecting the same trait is to be done in short time. Since gene pyramiding involves combinations of various desirable genes so it is impossible through conventional breeding.

However, adapting a resistant crop using gene pyramiding is the most desirable strategy to manage diseases [31]. Hence, MAS bridges the gap in crop development and also overcome big challenges and predictions of conventional breeding. In Africa, MAS has resulted in the recognition of quantitative trait loci (QTLs) that affects the productivity and nutritional quality of maize. Also many potential

Table 1. Molecular breeding for various traits in crop plants

S. No	Crops	Traits	References
1	Rice	Blast disease resistance	Li et al. 2019, Ning et al. 2020
		Genetic diversity	Hour et al. 2020; Hassan et al. 2022
		Nicotinamide synthase gene and Ferritin Gene	Boonyaves et al. 2017
		Yield enhancement	Gaikwad et al. 2014
		Bacterial blight resistance	Pradhan et al. 2015; Kumari et al. 2020
2	Wheat	Rust resistance genes	Yang et al. 2017; Babu et al. 2020
		Iron and zinc concentration	Wani et al. 2022
		Osmotic stress tolerance gene	Yang et al. 2016
3	Maize	Downy mildew resistance genes	Lohithaswa et al. 2015
		Provitamin A	Zunjare et al. 2018
4	Sunflower	Drought resistant genes	Hussain et al. 2017
		Heat and Drought stress genes	Killi et al. 2017
5	Finger millet	Salinity gene	Singh et al. 2022
6	Soybean	Seed oil content	Yao et al. 2020

Table 2. Advantages and disadvantages of various techniques used in molecular breeding

Techniques	Advantages	Disadvantages
Forward and reverse mutagenesis	Alternation in protein by the mutant allele	Sometimes deleterious modifications as it is not a targeted approach
Genetic diversity	Strengthens the ability of populations to resist biotic and abiotic stresses	Accumulation of number of deleterious genetic variations, which may increase the risk of extinction of a population
Marker Assisted Selection	Increases the efficiency for breeding as compared to conventional breeding	MAS is more expensive than conventional techniques
Gene introgression from wild relatives	Wild plants are source of desirable traits for disease resistance, fruit quality, and abiotic stresses	Linkage drag resulting in transfer of undesirable genes
Biofortification	Increase in food productivity and quality	Over-consumption of the nutrients
Genetic transformation	Increased food supply with less cost and longer shelf life	Addition of new allergens in food
Next Generation Sequencing	Generation of genomic resources in plants and decoding of a species genome	To differentiate various paralogous genes and pseudogenes
Genome editing	Crops are designed to make them more resistant to pests, reduction in pesticide use	Chance of errors is more during the gene editing process

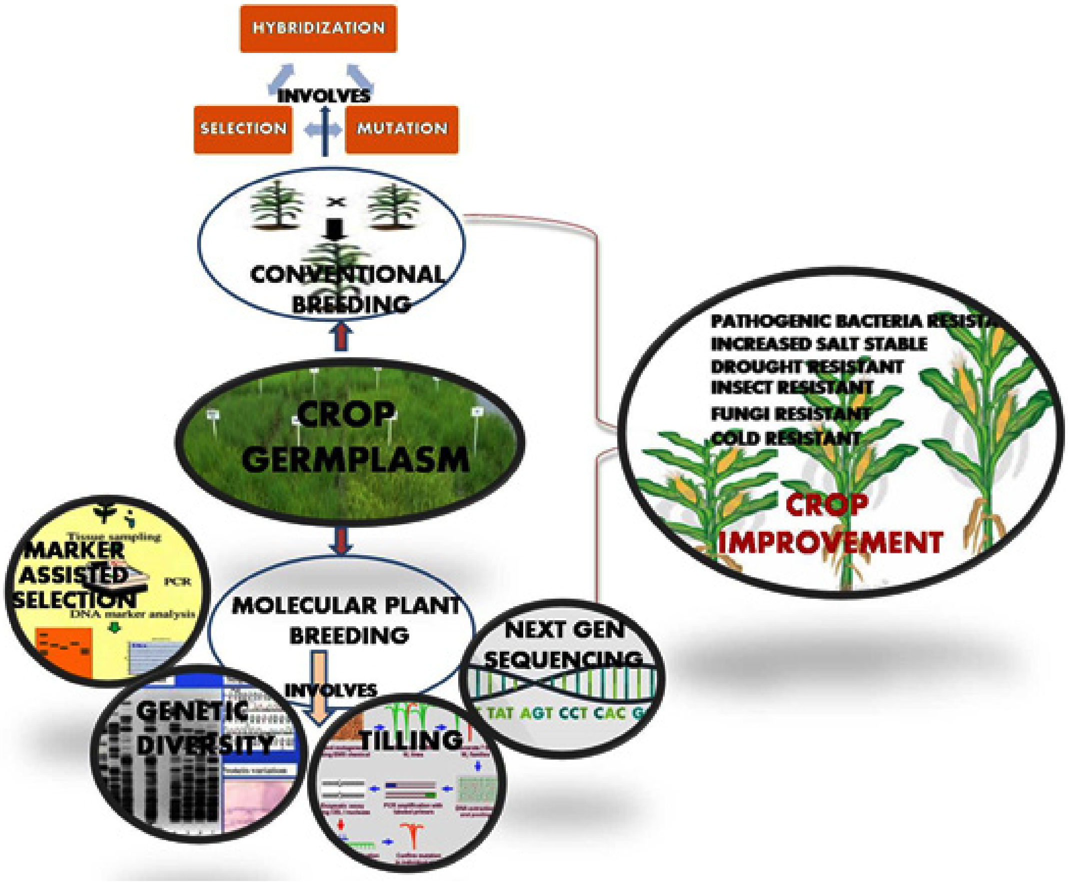

Fig. 1. Various approaches to molecular breeding in plants

QTLs linked to good agronomic traits have been mapped which show better performance in MAS breeding programs [32].

Diversification of gene pool using exotic germplasm

Widening the gene pool using exotic sources, can be challenging, as it leads to the introduction of many unwanted traits/alleles. Breeders have successfully used exotic germplasm for harnessing the germplasm by introducing the potential alleles associated with several desirable traits. The choice of the donor parents is prime factor to introgress alleles for specific attributes that can increase the crop productivity otherwise linkage disequilibrium can have deleterious effect [33]. Similarly, use of allied species can result in sterility of hybrids and linkage drag during interspecific hybrid production, [34]. In *Brassica* the elite breeding lines are recommended for canola cultivar development that has high-oleic acid content [35]. This makes the identification of right allelic combinations a difficult task. Sequencing of *Brassica* genome has facilitated the discovery of favorable alleles and their introduction into existing breeding material using biotechnological tools [36]. Similarly, on analyzing the diversity of *B. napus* gene pools with identified but allied *Brassica* species, genetic differences in *B. napus* were observed and thus these new favorable alleles can be introduced by the breeders to create better allelic combinations in *Brassica*. Oilseeds Research Institute, Faisalabad developed a new variety called "Super canola" which is high yielding and disease resistant. This variety was developed from RBN-03052 (a local canola line of rapeseed which is high yielding) and an Australian rapeseed variety (Rainbow), that has low Erucic acid and Glucosinolates [37].

Introgression of genes from wild/distant relatives

Mostly in some crop plants, the wild relatives serve as donors of useful genes for the genetic improvement of their progeny [38,39]. For this the genetic value of wild relatives needs to be conserved and can be further enhanced by the implementation of new breeding techniques. Even the information gained from model plant species also promises in the conservation of these genetic resources and transfer of the favorable alleles to generate new crop varieties. However, the environment from time to time poses problems for the recognition of positive alleles by hampering the expression of these useful alleles. Even the transfer of alleles into breeding material for generating the new crop varieties is not cost effective process as several plants with undesirable characteristics need to be eliminated at early stages of development so that only a few crop lines remain for testing [40]. In sunflower, genes for cytoplasmic male sterility, herbicide tolerance and biotic stress resistance have been successfully introgressed from the wild relatives into the cultivated gene pool, without any decrease in oil content and its quality [41]. Similarly, in rice, genes for bacterial blight resistance have been transferred from wild rice *O. nivara* and *O. glaberrima* to its cultivated variety *O. sativa* [42,43] (Table 1). This transfer of new alleles/gene(s) from wild resources to cultivated varieties will help in broadening the genetic base of the crop plants in near future.

Biofortification for nutrition improvement

Molecular breeding being a cost-effective tool contributes to global food security by increasing yield and the nutritional value of forage and crop plants. Biofortification, which is a new strategy for the improvement of crop nutritional quality through breeding addresses micronutrient and vitamin B6 deficiencies worldwide especially in women and children below 5 years [44,45]. It involves the development of micronutrient-rich staple crops using traditional breeding and modern biotechnology tools. Recent surveys in India have unraveled the positive effects of biofortified maize and wheat on young children. The consumption of zinc-biofortified wheat in form of chappatis, flatbread and porridge by rural adults and children has increased their immunization against pneumonia and vomiting generally caused by conventional wheat products [46].

The Consultative Group on International Agricultural Research (CGIAR) is exploring the potential to enhance the bioavailablity of iron and zinc content in staple crops such as rice, wheat and maize [47,48]. Beasley et al. [49] enhanced iron and zinc content in the wheat grains by transferring rice nicotianamine synthase-2 gene to upregulate the biosynthesis of nicotianamine and 2-deoxymugineic acid. Quality protein maize, a biofortified maize was introduced by the International Maize and Wheat Improvement Center, CIMMYT due to mutation in the opaque-2 gene that resulted in enhanced levels of essential amino acids (lysine and tryptophan) which were earlier lacking in the proteins of maize endosperm and hence reduced malnutrition in young children [50]. Similarly, millets are an important source of energy next to cereals, due to the presence of high protein content, essential amino acids, minerals and vitamins in their grains. HarvestPlus (a Program of the Consultative Group on International Agricultural Research), has released millet with high iron content in India to overcome the iron deficiency. The group decoded the QTLs and the genes linked to protein quality in finger millet using comparative genomics [51]. Thus, enhancing the nutrient value of crops through biofortification is a cost effective approach and further needs institutional leadership along with public and private policies to achieve the goals.

Genetic transformation

Favorable nutrition plays an important role in human health and progression. Crop yield is severely affected due to adverse environmental conditions. Thus, a major goal is to find different ways to increase the productivity as well as nutritional value in crop plants. The main crisis in 21st century agriculture is to attain 70% enhanced crop productivity in coming years in spite of the foreseen unfavorable environmental conditions so as to sustainably feed human population [52,53].

Genetic engineering is a ray of hope for improving agricultural productivity. Using this technique, improvement in crop can be done for enhanced nutritional value, tolerance to stresses, and efficient use of soil nutrients and water. Several transgenic crops such as rice, wheat, maize, cotton, potato, canola and sugar cane are growing globally and countries like US and Brazil are their leading producers.

Transgenic crops with novel candidate genes such as stress-responsive NAC transcription factors in rice, Alfin-like gene from *Atriplex hortensis* in *Arabidopsis* against drought and salt stress provide a quick method to develop stress tolerant crop varieties [54,55]. Similarly, in wheat transgenic, NF-YA transcription factor related to low nitrogen and low-phosphorus-inducible was over-expressed which resulted in significant increase in nitrogen and phosphorus uptake [56] along with a second transcription factor that played a role in nitrogen signaling [57], thus resulting in improved grain yield.

Similarly, there are several approaches that can increase the gene expression levels by the random activation of endogenous genes using enhancers, have conferred new functions to the plants [58]. Studies have revealed that a random insertion of transcriptional enhancers into the genome using strong promoter can result in over-expression of a member of a gene family. This involves no interference from other gene families, thus resulting in a novel phenotype and characterization of functionally redundant genes [59].

Next-generation sequencing and gene identification

Recently, advances in high-throughput genome sequencing have dramatically augmented the capability to characterize the genetic variants present in the germplasm including minor crops [60]. In finger millet, high-throughput sequencing has resulted in the characterization of calcium sensing and its accumulation mechanisms across genotypes and also to untangle the physiological and molecular basis of salinity response among genotypes under greenhouse conditions [61,62]. There information enabled the identification and maintenance of beneficial alleles and better choices of parents for producing hybrids.

Next generation sequencing has transformed the field of plant breeding by developing highly polymorphic, cost effective and closely linked molecular markers for a target trait [63]. Also, it led to the development of genome wide sequence based markers, which are less costly, both for minor and non-model crop plants. This has also facilitated the development of genomic resources for characterization of diversity, gene discovery, evolutionary relationships and marker assisted breeding in orphan crops which have no prior information on its genomic sequence [64].

Genome Editing

Genome-editing tools along with nucleases that are site-specific have provided efficient targeted modification in plant systems [65]. Genome-editing techniques have been utilized globally as an effective plant breeding tool against large variety of crop species [66]. During early times, techniques like use of zinc-finger nucleases [67] and transcription activator-like effector nucleases [68] were used for editing, but recently development of clustered regularly interspaced short palindromic repeats (CRISPR)/Cas system) has re-organized the editing efficiency with a variety of potential applications that ranges from gene knockouts to the introduction of small indels causing frame-shift mutations or introduction of premature stop codons [69].

CRISPR technique apart from being simple, efficient, and cost effective has also ability to target multiple genes. The CRISPR cleavage requires a short synthetic guide RNA sequence (about 20 nucleotides) that binds to the target DNA and a Cas9 nuclease enzyme that cleaves 3–4 bases after the protospacer adjacent motif (PAM). The Cas9 nuclease is made up of two domains, RuvC-like domains and a HNH domain, and each domain cut one DNA strand [70]. CRISPR/Cas9 techniques have been used widely against several plant genomes and the majority of editing has been done in plant species such as *Arabidopsis*, rice and tobacco [71].

In rice, reports have revealed that CRISPR/Cas9 targeted mutation occurring in the ethylene responsive factor increased resistance to blast disease [72], while development of two knockout mutants of *OsSWEET13* enhanced its resistance to bacterial blight disease [73]. Multiplexed genome editing was also done in hexaploid wheat where genes, one for grain trait and two for disease resistance were targeted using three gRNAs and a single promoter and further the editing efficiency was tested in transgenics [74]. In maize, Zhu et al. [75] using maize promoter (U6 snRNA), edited phytoene synthase gene involved in carotenoid biosynthesis that resulted in white kernels and albino seedlings. Transgenic showed that only stable *albino* mutants were produced without any off-target sites edited. Similarly, Young et al. [76] used a three-step approach in maize where computational prediction was combined with biochemical detection. They studied that if promiscuous guide RNA is intentionally designed with mismatches and in case if one mismatch occurrs in the PAM proximal region then only predictable off-target edits could be observed and minimized.

Thus, CRISPR/Cas9 based genome editing technique quickly inserts useful traits and improves agronomic output. This technique can help in designing suitable crops that will adapt to the current environmental conditions and will feed the growing human population to overcome global hunger.

CONCLUSIONS AND FUTURE PROSPECTS

Promising food security along with providing healthy food is a key challenge of the present day. Plant breeding has made an outstanding development in crop improvement by combining molecular techniques with conventional breeding that has created a wealth of information which can be further exploited for genetic improvement of crop plants, though an understanding of the current barriers and appropriate solutions needs to be developed. Though, breeding allows the pre-selection of genotypes with the desired combinations of alleles before field testing but still genetic constraints like epistasis, linkage drag of unwanted alleles remains challenge for cultivar development. The final aspire of molecular plant breeding is to introduce desirable traits in crop plants through certain breeding methods like backcrossing and recurrent selection, thus reducing the time for conventional selection which is based exclusively on phenotype. Further, advanced biotechnological tools and the genomic resources are providing plant breeders with new techniques and methodologies. Also, the beginning of high-throughput sequencing and genotyping, along with molecular tools has proved to be a boon for developing and under developed countries for dissecting the complex traits that exists due to strong environmental influence in the staple crops. However, there is still need to develop strategies using modern genomics tools and breeding approaches in combination for the successful integration of desirable genes in crops against destructive pathogens, biotic and abiotic stresses for the production of food in the near future.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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