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Chapter

Breeding Maize for Food and Nutritional Security

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Abstract

Maize occupies an important position in the world economy, and serves as an important source of food and feed. Together with rice and wheat, it provides at least 30 percent of the food calories to more than 4.5 billion people in 94 developing countries. Maize production is constrained by a wide range of biotic and abiotic stresses that keep afflicting maize production and productivity causing serious yield losses which bring yield levels below the potential levels. New innovations and trends in the areas of genomics, bioinformatics, and phenomics are enabling breeders with innovative tools, resources and technologies to breed superior resilient cultivars having the ability to resist the vagaries of climate and insect pest attacks. Maize has high nutritional value but is deficient in two amino acids *viz.* Lysine and Tryptophan. The various micronutrients present in maize are not sufficient to meet the nutritive demands of consumers, however the development of maize hybrids and composites with modifying nutritive value have proven to be good to meet the demands of consumers. Quality protein maize (QPM) developed by breeders have higher concentrations of lysine and tryptophan as compared to normal maize. Genetic level improvement has resulted in significant genetic gain, leading to increase in maize yield mainly on farmer's fields. Molecular tools when collaborated with conventional and traditional methodologies help in accelerating these improvement programs and are expected to enhance genetic gains and impact on marginal farmer's field. Genomic tools enable genetic dissections of complex QTL traits and promote an understanding of the physiological basis of key agronomic and stress adaptive and resistance traits. Marker-aided selection and genome-wide selection schemes are being implemented to accelerate genetic gain relating to yield, resilience, and nutritional quality. Efforts are being done worldwide by plant breeders to develop hybrids and composites of maize with high nutritive value to feed the people in future.

Keywords: food, maize, molecular breeding, Nutritional security, stress

1. Introduction

Maize or corn (*Zea mays* L.) belonging to Gramineae family is grown all over the globe as an important annual cereal crop. It is grown as staple food crop in many parts of the world and stands third leading cereal after wheat and rice [1].

Maize is globally called as queen of cereals due to its higher yield potential as compared to rest of the cereals. USA stands first in maize production which alone contributes about 35% of the world's total maize production. In India, the highest producer of maize is Uttar Pradesh, however it is grown in almost all the states in India. U.P, Bihar, Rajasthan, M.P, Punjab, Haryana, Maharashtra, Andhra Pradesh, H.P, W.B, Karnataka, and Jammu and Kashmir are major maize producing states, jointly accounting for over 95% of the national maize production [2]. For animal feed, maize is commonly used. It is commonly processed into different product categories, such as cornmeal, grits, starch, pasta, tortillas, snacks, and cereals for breakfast. Flour of maize is used to make chapatis or flat breads that used to be common dishes in a few northern states of India [3]. The phytochemical compounds obtained from maize and their health properties have recently become the main focus of studies due to increasing attention to the development of nutraceuticals. Maize a monoecious plant has differently located male and female flowers. Tassel is the male flower, and silk is the female flower. Tassel develops 2–5 days before silk that makes it protandrous in nature. The cultivated maize has six different types with each one having different characteristics and specific use, (i) Dent corn: Mostly grown in USA, called as soft maize, (ii) Flint corn: Also called hard corn and is mostly grown in India, (iii) Sweet corn: sweet in taste due to both starch and sugar present in it. It is harvest green and fetch fresh to the market, (iv) Pop corn: most popular type of corn for consumption purpose in humans. It has small sized grains with hard endosperm, (v) Flour corn: the starch present in this type is very soft and the corn shrinks on ripening (vi) Waxy corn: this corn has a soft wax-like substance which resembles tapioca starch.

Maize for livestock feed is important both as residue of crops and silage, grain and is used for extraction of oil and starch industrially. The biological value of maize reflects that it is rich source of carbohydrate, protein, iron, vitamin B, and minerals. The fresh green cobs are eaten separately as roasted or boiled or mixed with legumes. The maize plant has economic value in its every part like the grain, leaves, stalk, tassel, and cob to produce different varieties of food and non-food products.

2. Maize: a potential nutritious cerealcrop

The nutrient status of kernels in maize depends on the genotype or genetic background, management of agronomic practices, genotype-environment interaction and handling after the harvest of produce [4]. Most of the micronutrients present in widely used maize are not sufficient enough to meet nutritional demand of consumers, however the concentration of different micronutrients can be increased by developing cultivars with improved neutraceticals due to higher degrees of genetic variations in maize. This process is called biofortification [5]. Additional or complementary crop management and food science innovations may also help to enhance the nutritional effect of diets based on maize [6]. Maize researchers have developed improved nutritionally useful cultivars such as quality protein maize (QPM) rich in lysine and tryprophan [7], biofortified orange maize with provitamin-A carotenoids [8] and high-Zn-enhanced maize [9]. Through conventional breeding, higher levels of lysine and tryptophan, kernel Zn and provitamin- A have been successfully increased in maize. Due to various factors, including the availability of large genetic diversity for the target characteristics, advances in understanding key biochemical pathways for metabolite biosynthesis, analytical tools for screening germplasm for quality characteristics, and the possibilities for understanding key biochemical pathways for metabolite biosynthesis, there are now significant

opportunities for more effective development of nutritionally enriched cultivars of both grain and specialty maize [10].

3. Requirements for breeding of maize

Maize is mostly grown as an energetic crop, but the use of various unique varieties is very extensive, such as high-oil maize, high-lysine maize, waxy maize, amylose maize, flint maize, white maize, popcorn and sweet maize. Unique types of traits need special attention in the selection and seed production process, as well as in the commercial production process. While breeding principles are the same for all types of maize, the approach to selection for each particular type is very much different. Due to their characteristics and genetic regulation of these characteristics, specific types of maize need special care in the handling of breeding materials during breeding processes. It is particularly associated with preventing uncontrolled pollination. Specific characteristic assessment procedures, such as estimation of popping volume and flake consistency in popcorn, determination of sugar and harvest maturity in sweet maize, determination of oil in selected samples of high-oil maize varieties, etc., are important to ensure good selection of a certain characteristic. Since the recent past, a large number of high-yielding hybrids of sweet corn, popcorn, high-oil and high-lysine, flint and white maize have been grown. The abundant genetic diversity and technical and technological possibilities required for good selection promote auspicious selection and breeding for these characteristics.

4. QPM: an alternative to normal maize

Quality protein maize (QPM) was developed in the late 1960s [11] and produces 70–100% more lysine and tryptophan than ordinary modern and traditional tropical maize varieties [12]. In addition, QPM nutritional assessment in different locations has demonstrated the consistency of the content of lysine and tryptophan within the recommended range for QPM, considering very diverse types of environmental conditions [13]. In QPM grain, the nutritional content of the protein exceeds that of cow's milk protein. In developing countries, the adoption of QPM will significantly contribute to alleviating malnutrition in maize-based economies [14]. For example, substituting normal maize in stock feeds has been found to be of economic benefit because it needs small amounts or no supplemental protein sources to balance the diet [15]. QPM cultivars may be competitive in productivity with normal maize and should demonstrate stable performance across environments, especially in terms of yield and protein quality characteristics [13]. Combining high yield with high-quality protein content in an elite maize variety has been a great challenge. QPM development faces severe biotic (diseases and pests) and abiotic (drought, heat, low soil pH, low soil nitrogen, etc.) limitations, as with normal maize. Several studies have been performed around the world to alleviate some of these limitations on breeding for QPM resistance to pests and diseases [16] and tolerance to abiotic stresses such as drought, low soil nitrogen, heat stress and combined heat and drought stress [17]. Quite a number of QPM studies have been performed on improving the nutritional performance and disease tolerance of QPM in breeding programs around the world in recent years [18]. The main research emphasis has been on growing the protein content and exploring genetic variability between QPM genotypes and normal endosperm maize varieties. More research, however, needs to be placed on the resistance of QPM varieties to certain abiotic

stresses, such as heat stress, dryness and heat stress combinations, and low soil pH. In Africa, malnutrition is a persistent issue, especially in rural areas where poor people depend on staple foods and have limited access to a diverse diet. Bio-fortified crops bred for enhanced nutritional quality may mitigate nutritional deficiencies if adequate quantities are produced and consumed. The positive effect of QPM on the nutritional status of human consumption and animal feed has been shown by several studies in controlled settings [19]. In order to preserve protein content in grain, lysine or tryptophan levels should be continuously monitored during the breeding period, even if the op2op2 genotype is retained. One way to solve the problem of malnutrition in the world is the consumption of QPM varieties, especially for people who are low in resources and cannot afford other sources of protein for their families. QPM may also be used as an additional food for humans (particularly pregnant women, lactating mothers and young children) and for animal feeding. Moreover, for refugees and other people facing nutritional challenges around the world, QPM flour can also be very useful.

5. Breeding approaches for QPM

It was introduced into many breeding programs worldwide after the discovery of the nutritional benefits of the opaque-2 (op2) mutation, with a significant focus on the conversion of normal endosperm populations and inbred lines to op2 versions via a modified backcrossing-cum-recurrent selection process. At CIMMYT, QPM breeding strategies concentrate on pedigree breeding, whereby the best performing inbred lines, complementary in various characteristics, are crossed to create new segregating families. New inbred lines are formed from these segregating families [20].

5.1 Conventional and molecular breeding approaches in QPM

Pixley and Bjarnason [21] reported that the consistency of proteins was very stable across environments, while QPM varieties were less stable in protein content and endosperm modifications. Pfunde and Mutengwa [22] reported that early maturing QPM inbred lines under drought stress could be used in a breeding programme as sources of early maturation, whereas early maturing single crosses could potentially be recommended in drought-prone areas for maize growers. The stability performance of CIMMYT tropical and subtropical elite QPM hybrids across stressed and non-stressed environments was analyzed [13]. In drought conditions with wide variability in grain yield and protein content among genotypes, the stress effect was comparatively large, indicating that the content of tryptophan and lysine is most stable across stressed and non-stressed environments. While drought tolerance screening has largely been performed for QPM varieties for the vegetative to flowering stages of development, very few studies have been done on tolerance to early drought stress at the seedling level. Drought tolerance has often been hated at the seedling stage of development in that it does not offer an indicator of a genotype's yielding ability under drought stress. Clearly, therefore, the correlation parameters that could relate early drought response to late drought stress tolerance need to be further investigated.

Henry *et al*. [23] studied the molecular structure of the opaque-2 gene and found that the molecular diversity in the transcriptional activator op2 was very high relative to that of other maize transcription factors. Multiple genes have been identified to regulate the quality of amino acids. In order to monitor the levels of a protein synthesis factor associated with lysine levels, at least three loci were involved and

these were mapped on chromosomes 2, 4 and 7 [24]. Via marker-assisted backcross breeding, two QPM lines (CML 180 and CML 170) were selected as donors for introgression of the op2 allele into regular maize inbreds (CM 212 and CM 145) because the crosses between the donor QPM lines and non-QPM lines showed a 41% increase in tryptophan and a 30% increase in lysine over the original hybrid lines [23]. Therefore, modified marker-assisted back cross breeding is a potential way to produce QPM variants of standard maize inbreds with suitable endosperm features that can be combined to create QPM hybrids. Using inter-simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) markers. Nkongolo *et al*. [25] studied the degree of genetic variation and relatedness among and within QPM and non-QPM varieties. The findings showed that the genetic difference between QPM and non-QPM varieties and within them was high, while the genetic gap among them was minimal, giving the possibility of developing improved QPM hybrids. The use of molecular markers in QPM breeding programs shortens the selection process, making it more effective across environments during the production of enhanced genotypes. It is important to remember that it is considered that the latest generation of markers such as SNPs is comparatively more efficient and cheaper than older models (SSR, RAPD).

In CIMMYT, several QPM populations, inbreds, hybrids and pools were developed through conventional conversion breeding methods that could adapt to subtropical and tropical environments and are widely used in the production of QPM cultivars in several countries in Africa, Asia, and Latin America [26]. Two measures are involved in marker-assisted introgression using backcross breeding: (1) foreground selection: targeting gene by marker, and (2) background selection: targeting uniformly distributed markers for recurrent parental genome (RPG) recovery across the genome [27]. This is an effective way to transfer particular gene(s) to an otherwise superior variety or parental lines. By foreground selection, the detection of the gene of interest becomes accurate, while background selection accelerates the rate of RPG recovery with two backcrosses [28]. Simple access to accurate gene-based or linked markers based on PCR has made MAS an effective alternative. Microsatellite or Simple Sequence Repeat (SSR) markers are often the choice for their low cost, simplicity and effectiveness among the various types of DNA sequence-based markers available. Codominant, stable, hypervariable, abundant and evenly distributed SSR markers are distributed throughout plant genomes [29]. Several thousand SSRs in maize are mapped and accessible in the public domain. The availability of sufficient linked SSRs has provided a promising choice for marker-assisted introgression of o16 to further enhance the nutritional quality attributes of grain, in particular lysine and tryptophan in endosperm protein. In this context, associated SSRs, umc1141 and umc1149, were successfully used for introgression or pyramidization of o16 alone in the genetic context of o2. The improvement of the quality of proteins (tryptophan and lysine) by o16 over normal maize is comparable to the QPM genotypes based on o2 [30]. At Guizhou Institute of Upland Food Crops, Guizhou Academy of Agricultural Sciences, China, Marker Assisted Selection (MAS) was used to improve parental lines and derived hybrids by pyramiding o2 and o16 in maize adapted to temperate regions. A half-fold increase in lysine content has been reported among pyramid progenies of o2 and o16 [31].

6. Lysine: potential source for food security

Due to breeding of modern maize hybrids for higher yields at the cost of protein, the grain composition has inadvertently trended to higher starch content [32]. In addition, as corn grain protein is deficient in some amino acids that are nutritionally

important, this decline in the amount of grain protein has further decreased the grain's nutritional quality. Increasing the nutritional quality of maize grain protein, particularly by increasing the content of essential amino acids, such as lysine and tryptophan, is one approach to addressing this issue.

With regard to the nutritional needs of monogastric animals, the most restrictive amino acid in corn grain is lysine. Improving the content of lysine is therefore a primary goal for improving the quality of maize grain. Maize protein's low nutritional content is mainly affected by the amino acid composition of endosperm proteins. Corn protein has a 2.7 percent lysine content, which is slightly below the FAO recommendation for human nutrition. While the germ protein in the whole grain has a sufficient lysine content (5.4%), this is diluted by the far more abundant endosperm proteins, which have an average lysine content of only about 1.9%. This is because 60–70% of endosperm protein is made up of zeins that contain little to no residues of lysine [33]. Likewise, the lack of residues of tryptophan in zein proteins is the explanation for the low content of corn protein in tryptophan. Changing the profile of the grain protein through approaches such as zein reduction and lysine-rich protein expression could therefore significantly boost the amino acid balance. Alternatively, by elevating the free lysine level in the kernel, the lysine content of the grain could be increased.

There is overwhelming evidence available showing QPM's nutritional dominance over standard maize. Different QPM feeding studies have been performed where under-nourished children given QPM as the only source of protein showed the same growth as those given modified cow milk formula in the diet [34]. Independent research in various countries reported a 12 percent rise in weight in children eating QPM over traditional maize [35]. A study conducted in Guatemala found that the nutritional value of o2 maize is 90% of milk protein compared to 40% of regular maize in young children [11]. QPM has other nutritional advantages, i.e. a stronger leucine/isoleucine ratio and greater niacin availability, with a double increase in tryptophan and lysine and a doubling of biologically functional protein [36]. Even though QPM and normal maize have the same niacin levels, the low leucine content in QPM helps to release more tryptophan for niacin biosynthesis. Thus, pellagra is substantially reduced by QPM [26].

Several animal feed experiments were also performed to test QPM's nutritional benefits and biological superiority. It was first seen in rats where a threefold increase in growth rate was observed when fed a 90% QPM diet. Rats fed with the QPM diet weighed more and were thicker, longer, denser and stronger than ordinary maize diets [37]. The nutritional benefits of QPM have also been systematically carried out in pigs. In pigs raised on QPM, the weight gain was doubled compared to those feeding on only standard corn [38]. Pigs fed with a QPM diet alone with supplements of vitamins and minerals increased twice the rate of normal maize fed by pigs [39].

7. Provitamin-a-biofortified maize (PVABM): future food

One of PVABM's benefits is that it is cheaper than other vitamin A supplements [40]. There is a lower production cost in subsequent years after the crops have been bred and grown, given the necessary storage conditions. In addition, there is no need for additional fortification or vitamin modifications in people's diets once maize has been produced at the farm level [41]. Staple crops, such as maize, are used in rural communities to prepare various meals, so changes in nutrients can stabilize the nutrient composition within them [42]. Under smallholder farming systems, biofortification targets staple crops [43]. To improve the acceptability and

accessibility of vitamin A at the household level, various maize products can be developed through PVABM. In rural communities, where maize is used for various goods, the production of PVABM can boost the local economy by people selling snacks, and can improve food security by allowing different meals to be eaten at different times, resulting in decreased VAD in children. There is no doubt that PVABM will boost the food security status of rural households and alleviate VAD, but the willingness of smallholder farmers to embrace PVABM and the acceptability of these products by consumers is a challenge before it can be integrated into smallholder farming systems. Yellow maize is commonly confused with orange maize by rural populations, which could be a major challenge given the perceptions surrounding yellow maize. Across the African continent including South Africa, PVABM has drawn attention from researchers in various fields [44]. In rural areas, where the target groups are mostly located, PVABM has the potential to alleviate VAD, hidden hunger, and boost food security. In order to fix VAD, the carotenoid content in PVABM is essential.

7.1 Carotenoids in PVABM

In the form of provitamin A, maize grain produces various forms of carotenoids [45] and are present in yellow and orange maize. The carotenoid pigments present in yellow and orange maize result from xanthophyll and carotenes, and are responsible for the endosperm color (yellow or orange). In PVABM, the most abundant carotenoids have been described as β-carotene and β-cryptoxanthin, while alpha-carotene is present in smaller capacities. The amount of carotenoids increases with the change in color [46]. Dark orange maize has higher carotenoid levels than other colored maize, but orange and dark orange maize are still not available to farmers and consumers.

8. Genomics-assisted breeding

Genomics-assisted breeding (GAB) for crop improvement initiates with identification of genomic markers linked with QTL or gene(s) related to the target trait and then the application in the breeding platform. Various GAB strategies have been used in crop improvement, including marker- assisted backcrossing (MAB), marker-assisted recurrent selection (MARS), and genomic selection (GS). Recently, speed breeding is included to the list.

8.1 Marker-assisted backcrossing and recurrent selection

Marker-assisted backcrossing (MABC) is the introgression of a genomic region (QTL or locus or gene) contributing the desired trait from a donor genotype into a breeding line or elite cultivar without linkage drag through back- crossing after multiple generations. The resultant product of MABC contains the whole genome of an elite parent with the genetic loci or QTL or gene(s) contributing to the desired phenotype from the donor parent [47]. Quantity of molecular marker used, the strength of marker association with the phenotype, undesirable linkage drags, and size of the population used for each generation of back- crossing determines the efficiency of MABC. This method has been used extensively to generate superior lines of varieties for biotic and abiotic stress tolerance. The marker-assisted recurrent selection (MARS) was introduced to counter the inefficiency of MABC in transferring multiple QTLs regulating complex traits like yield or broad-spectrum disease resistance. MARS involves the detection and selection of large QTLs or

multiple genomic regions controlling complex agronomic traits within a single or across the populations and their pyramiding in a single genotype [48]. This approach makes use of the F_2 population and is most effective for cross-pollinating species. In disparity with MABC, favorable alleles may be contributed by both the parents, and the selected improved genotype becomes the chimera of their parents. The superior allele enrichment involves the phenotypic and marker effect for desired traits in the F_2 population, followed by two or multiple cycles of marker-assisted selection [49]. In the past few years, the Hyderabad situated International Maize and Wheat Improvement Center (CIMMYT) has made significant headway in the development of drought-tolerant maize inbred lines through MARS approach in their Asia Maize Drought Tolerance (AMDROUT) project.

8.2 Genomic selection and speed breeding

Genomic selection (GS) or genome-wide selection (GWS) employs large-scale DNA markers throughout the genome for developing superior germplasm lines. The genomic selection approach has the potential to express multiple QTLs/ genes which are widely distributed throughout the genome. Vigorous phenotyping is not necessary to develop a breeding population, and subsequent offspring selection is based on genotypic predictions, which combines both the genomic and pedigree data for several generations of the breeding cycle [50]. The sum of the information index with a combined effect of genome wide molecular markers called the Genomic estimated breeding value (GEBV), is the basis of recurrent selection [51]. High- density molecular markers where each QTLs is in linkage disequilibrium with a marker is necessary prerequisites for precise GEBV, and thus, for GWS. The success of GS mainly depends on the quantity and diversity of the training population (breeding lines selected for the GWS programme). The reduced number of selection events has decreased the time and cost of breeding. While breeding crops and releasing cultivars for farmers, time is a critical factor as normally it takes 3–7 years for crossing experiments, followed by long evaluation for yield, diseases and quality, and varietal release. Therefore, the approach of modulating day-light and duration for increasing the life cycle, term 'speed breeding', has been introduced. It shortens the breeding cycle by accelerating crop generation in glass- houses and growth chambers by providing controlled rapid growth-promoting conditions [52]. By balancing factors like photoperiod, humidity, temperature, and others we may achieve six generations per year for crops like wheat, barley, canola and chickpea [53]. Also, in the glass-house, these crops undergo only three generations in a year [53]. Early anthesis in plants was reported grown under speed breeding with fully viable mature seeds with unaffected yield between speed breeding and normal photoperiod conditions in almost all crops [52]. This programme accelerates the generations in mapping population as compared to the duration of MABC/ MARS/GWS, and accelerate the progression towards homozygosity. It has been used in all major crops (annual or biannual), and even in woody shrubs or perennial crops. Reduction of juvenile phase from 5 years to 10 months in apple and 7 to 2 years in chestnut are some of the example of the application of accelerated breeding cycle in perennial crops [54]. Rana et al., [55] has combined marker-assisted selection with speed breeding for developing salt-tolerant rice lines. Jighly et al., [56] coupled Genomic Selection with speed breeding to enhance genetic gains in allogamous plants for example tall fescue. The approach named Speed GS is gaining popularity among breeders for achieving higher genetic gain per cycle, especially for traits with low heritability.

9. Biotechnological interventions

Genetically modified (GM) or transgenic crops have modified genomes achieved through several genetic engineering techniques. Conventional plant breeding is time-consuming and enables the transfer of genetic information from closely related species, genetic engineering facilitates gene transfer across barriers from any source. With the help of established protocol for introducing gene into host species plus a rigorous selection method is needed for greater success. *Agrobacterium tumefaciens*-mediated genetic transformation is among the most reliable approaches being used for achieving stable transgenic lines. Other techniques, like particle bombardment (biolistics), sonication, and electroporation, are used for transient expression of the foreign DNA. As of now 525 transgenics in 32 crops have been commercialized, of which *Zea mays* holds the highest rank. Transgenic crop cultivation enhances agricultural productivity to about 22% leading to a 68% increase in profits [57].

10. Candidate genes

Gene cloning and isolation facilities help introgression of a target gene from any genome which is transformed into any other genome for its desired expression. Most historical example is of expression of 'Cry' gene of *Bacillus thuringiensis* for overcoming he hazards of pests and insects attack. DREB (dehydration responsive element binding) protein-encoding genes are a class of genes that are frequently isolated from one species and expressed in another for enhancing the resilience and tolerance towards different abiotic and biotic stresses. By the advancement of NGS technologies, expression and overexpression strategies also assist in illuminating the gene function, which is otherwise a useful task in covering huge genes amounts. The functional gene characterization is required to utilize the gene in developing stress tolerant plant cultivars by overexpression of candidate genes for example, T-DNA insertion lines of *Arabidopsis thaliana* have helped in understanding gene function. Overexpression of *ARGOS* genes in maize (*Zea mays* L.) leads to a reduction in sensitivity to ethylene, and transgenic plants show enhanced drought resistance as well as higher grain yield in well-watered as well as drought conditions [58]. Genome sequence information has facilitated the large-scale gene analysis, characterizing genes for their agronomic, physiochemical and other traits, genomic composition, promoter elements and expression profiling of genes towards stress which have helped in identifying candidate genes.

11. RNA interference

Also known as co-suppression, post-transcriptional gene silencing (PTGS). It is a biological process where RNA molecules inhibit gene expression or translation, by neutralizing targeted mRNA molecules. Its discovery is a breakthrough in the history of biology, and it has been widely utilized in functional genomics, reverse genetics and crop improvement [59]. RNAi pathway involves the generation of small RNAs (sRNA), which include short interfering RNA (siRNA), microRNA (miRNA), transacting siRNA (ta-siRNA) and natural-antisense siRNA (NAT-siRNA) which mediate silencing or epigenetic regulation of their target genes. Transformative RNAi has been used in several modified forms like artificial miRNA (amiRNA), artificial ta-siRNA (ata-siRNA), hairpin RNA (hpRNA), intrinsic direct

repeat, 3′-untranslated region (UTR) direct repeat, terminator-less, single-stranded promoter antisense and intron delivered promoter hpRNA [60]. Significant examples include alteration of plant architecture, improvement in β-carotene and lycopene content in fruits, good shelf life and nutritional improvement like low gluten content, reduction in toxic terpenoids, biotic stress resistance against viruses, fungi, bacteria and nematodes; and abiotic stress resistance [61]. The non-transformative RNAi technique, spray induced gene silencing (SIGS), has gained widespread acceptance as it is easy to use and has low cost of application. It works by spraying plants with double-stranded (ds) RNA/siRNA and has been successfully utilized for controlling insect pests [62]. Plants sprayed with dsRNA/ sRNA targeting *DCL1* and *DCL2* of *Botrytis cinerea* showed a significant reduction in gray mold disease symptoms showing the use of this technology for the developing eco-friendly bio-fungicides. Transgenic plants are still not accepted in many countries and it is estimated that about 130 million dollars exhausted on commercializing a transgenic crop [59]. SIGS being a non-GMO approach has enormous prospective for crop improvement.

12. Gene and genome editing

Genome editing (also called gene editing) is a group of technologies that give scientists the ability to change an organism's DNA. These technologies allow genetic material to be added, removed, or altered at particular locations in the genome. Several approaches to genome editing have been developed. A recent one is known as CRISPR-Cas9, which is short for clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9. The CRISPR-Cas9 system has generated a lot of excitement in the scientific community because it is faster, cheaper, more accurate, and more efficient than other existing genome editing methods. Precise genome editing started when for the first time, it was seen that DNA binding zinc finger domains along with Fok1 endonuclease domains could cleave DNA at defined regions and act as site-specific nucleases (SSNs) [63]. Further research led to the development of transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindrome repeats (CRISPR)/CRISPR-associated protein 9 (Cas9). Meganu- cleases (MegaN) recognize long DNA sequences that are greater than 14 nucleotides (nt) up to 40 nt. Since they have endonuclease activity, they produce double-stranded (ds) breaks at the recognition sites [64]. CRISPR/Cas9 is easy to use and is therefore, more popular compared to other genome editing technologies [65]. CRISPR/Cas9 comprises of two components: a single-guide RNA that is customizable and Cas9 endonuclease. Protospacer adjacent motif (PAM) (5′NGG3′) is a prerequisite needed for inducing ds breaks at the targeted sites in the genome. The breaks are repaired through either homology directed repair (HoDR) or non-homologous end joining (NHEJ). Since NHEJ is error- prone, repair leads to insertions or deletions at the target site. CRISPR/Cas9 has shown promising results for crop improvement, and for several nutritional traits and biotic and abiotic stress resistance [65].

Technologies like molecular breeding and genetic manipulation help to achieve food security and resilience to various biotic and abiotic stresses. Advances in NGS technology have enabled the incorporation of genomics with various disciplines of crop breeding. Large-scale genomic markers and high-throughput genotyping have accelerated improved cultivar development in terms of cost and resources. Functional and comparative genomics have provided the platform for gene discovery and gene functional characterization. The key gene or genes regulating a molecular pathway are being genetically engineered to breed phenotypically

improved crop lines. Conventional approaches together with biotechnological tools aim to increase productivity per plant and minimize yield loss at the farmer's level. The collaborative research investments in both the approaches are indispensable to food security and sustainable crop improvement.

Future thrust areas

- Discovery of trait specific novel genes from maize genomes
- Delivering superior single cross hybrids with diverse genetic base for various segments
- Thoroughly integrating marker assisted selection and doubled haploids in breeding programs
- Spearheading development of public sector events of transgenic maize
- Inventing new generation of ecofriendly and bio safe technologies for maize value chain
- Developing and popularizing high yielding, profitable and ecologically sustainable maize based farming systems
- Precision input management for higher productivity, profitability and environmental sustainability
- Popularizing resource conservation technologies in maize systems

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