

Hybrid Development and Seed Production of Biofortified Maize: Aspects and Prospects

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ABSTRACT: Malnutrition affects a large section of the population worldwide. It not only affects growth and development in humans but also causes socio-economic imbalance. Among various approaches, crop-biofortification has emerged as a sustainable and cost-effective tool to alleviate malnutrition. Intensive research efforts in India and abroad have led to the development and release of a series of biofortified maize hybrids for commercial cultivation. The quantity of essential amino acids, viz. lysine and tryptophan, provitamin-A, and vitamin E has been enhanced, while phytate has been reduced to improve the bioavailability of iron and zinc. This has led to the development and release of several multinutrient-rich maize hybrids. Availability of markers for *opaque2*, *crtRB1*, *lcyE*, *vte4*, *lpa1*, and *lpa2* has helped in integrating molecular breeding into the biofortification programme. Besides, new traits such as anthocyanins, methionine, and oil have also been enhanced in an elite genetic background. We discuss here the status of the biofortification programme of maize in India, coupled with the seed production system prevalent in maize with special reference to biofortified inbreds and hybrids. Specific requirements of breeder-, foundation-, and certified- seed in India to ensure the supply of quality seeds have also been presented. The potential of novel genetic systems for the rapid development and popularization of biofortified hybrids has also been discussed.

Keywords: Nutrition, corn, protein quality, vitamins, minerals, seed, biofortification

Status of malnutrition

Malnutrition caused by unbalanced food intake affects human growth and development and hampers socio-economic progress [1, 2, 3]. An estimated 733 million people are hungry, while 2.8 billion cannot afford healthy food worldwide [4]. Globally, 148.1 million (22.3%) children under 5 are stunted, 45 million (6.8%) children are wasted, and 37 million (5.6%) children are affected by obesity [5]. Anaemia is also widespread, affecting 57% of adult women and 25% of adult men in India [6]. Malnutrition in all forms costs society about US\$3.5 trillion annually [7]. Among various strategies, 'crop biofortification' has become the most promising approach to reduce micronutrient malnutrition [8]. Compared to other methods, biofortification offers advantages such as sustainability, cost-effectiveness, and providing nutrients in their natural form [9]. Maize has become the most important cereal crop globally, serving as food, feed, and a range of industrial products [10, 11]. In India, maize grains are mainly used as poultry (47%) and livestock feed (13%), with its popularity as food (13%) and processed food (7%) [12]. Additionally, maize is used in

starch industries (14%) and exported (6%). However, traditional maize has poor nutritional qualities, including low levels of protein quality (lysine, tryptophan, and methionine), provitamin-A, vitamin-E, anthocyanin, iron, and zinc [13, 14]. With the advancement of molecular technology, several biofortified maize hybrids have been developed globally through accelerated breeding [15].

Molecular breeding has emerged as a popular choice among plant breeders [16]. While conventional breeding takes 10-15 years to develop a suitable cultivar, molecular breeding accelerates the breeding cycle and helps to develop cultivars in less than half of the time [17]. Further, molecular breeding ensures selection of target gene(s)/ QTLs with more precision, besides selecting desirable plants at the seedling stage, much before expression of the trait(s). Costly and cumbersome phenotyping can be bypassed, and linkage drag is also avoided in molecular breeding [16, 18]. The development of dense molecular genetic maps and the availability of robust molecular markers have made application in marker-assisted selection (MAS) easy and straightforward [15]. Maize is

one of the earliest crops where MAS was applied in breeding [19]. Molecular breeding is now widely applied in the improvement of nutritional quality traits in maize [20]. The genetic value addition for major nutritional traits in maize through molecular breeding is presented below.

Development of biofortified maize hybrids

Protein quality

Maize protein is deficient in lysine, tryptophan, and methionine [21, 22]. Lysine and tryptophan serve as precursors for several neurotransmitters and metabolic regulators, and their deficiency leads to reduced appetite, delayed growth, impaired skeletal development, and aberrant behaviour in humans [23]. The recessive allele of the *Opaque2* (*O2*) gene located on chromosome-7 causes doubling of lysine and tryptophan [24]. Several *o2*-based Quality Protein Maize (QPM) hybrids have been developed through conventional breeding [25]. However, with the advent of molecular markers, several QPM hybrids have been developed through MAS. ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan (VPKAS), Almora, developed 'Vivek QPM9' during 2008 [26]. It is the country's first MAS-derived maize hybrid. Later, three more QPM hybrids, viz., 'Pusa HM-4 Improved' (2017), 'Pusa HM-8 Improved' (2017), and 'Pusa HM-9 Improved' (2017), were developed through MAS by ICAR-Indian Agricultural Research Institute (IARI), New Delhi [17] (Table 1). Besides, MAS-derived QPM hybrids, 'VL QPM Hybrid-45' (2022) developed by VPKAS, and 'QPMH-6' (2024) developed by ICAR-Indian Institute of Maize Research (IIMR), Ludhiana, were also released for commercial cultivation.

Provitamin-A

Vitamin-A is essential for normal body function, such as proper vision, maintenance of cell function, epithelial integrity, red blood cell production, immunity, and reproductive systems [27]. Though yellow maize possesses tremendous natural variation for carotenoids, it is predominantly of lutein and zeaxanthin [28]. Provitamin-A carotenoids are present with <2 ppm as compared to the target level of 15 ppm [29]. Two genes, *lycopene epsilon cyclase* (*lcyE*) on chromosome-8 and *β-carotene hydroxylase* (*crtRB1*) on chromosome-10, have been shown to regulate the accumulation of provitamin-A compounds [30, 31]. Provitamin-A rich hybrids, 'Pusa Vivek Hybrid-27 Improved' (2020) and 'VL

Vita' (2023) have been developed by IARI (Table 1) and VPKAS, respectively [32]. These hybrids have been developed through molecular breeding.

Vitamin-E

Vitamin-E or tocopherol is an essential micronutrient for reproduction and quenches free radicals in the cell membrane. It protects humans from cardiovascular disease, Alzheimer's disease, neurological disorders, and many age-related degenerations [33]. Maize kernels are rich in total tocopherol, of which γ -tocopherol constitutes ~80% and α -tocopherol accounts for ~20% of the total pool. Due to favourable interaction with the receptor, α -tocopherol is present 10 times more than γ -tocopherol in the plasma of humans. A favourable allele of *vte4* (chromosome-5) coding *γ-tocopherol methyl transferase* (γ -TMT) accumulates α -tocopherol by 3.2-fold [34]. In India, Pusa Biofortified Maize Hybrid-5 (2024) and Pusa Biofortified Maize Hybrid-6 (2025), possessing high α -tocopherol have been developed through MAS by IARI [20, 35]. (Table 1).

Iron and zinc

Humans require iron for basic cellular functions and proper functioning of the muscle, brain, and red blood cells [36]. Zinc is an essential mineral for humans, animals, and plants for many biological functions. It plays a crucial role in more than 300 enzymes in the human body for the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids [37]. Phytic acid (PA) (*myo*-inositol-1, 2, 3, 4, 5, 6-hexakisphosphate or InsP6) is a ubiquitous and the most abundant inositol phosphate found in all eukaryotic cells. Owing to negative charge, PA are potent chelators of nutritionally important positively charged mineral ions, viz., iron and zinc. Extensive research in seed phytic acid content has led to the isolation of three *lpa* mutations in maize, namely *lpa1*, *lpa2*, and *lpa3*, possessing 66%, 50%, and 50% less phytic acid compared to wild-type alleles, respectively [38, 39]. 'PMH-1-LP' with *lpa2* (chromosome-1) is a low phytate maize hybrid developed by IIMR, Ludhiana, and later released in India during 2022. While low phytate maize hybrids, 'Pusa Biofortified Maize Hybrid-7' (2025) and 'Pusa Biofortified Maize Hybrid-8' (2025) with *lpa1* (chromosome-1) developed by IARI have been released for commercial cultivation (Table 1). These low-phytate maize hybrids have also been developed through the MAS strategy.

Table 1. Details of MAS-based biofortified maize hybrids released by IARI

S. No.	Name of the hybrid	Nutritional trait(s) improved
1.	Pusa HM-4 Improved, Pusa HM-8 Improved & Pusa HM-9 Improved	QPM
2.	Pusa Vivek Hybrid-27 Improved	PVA
3.	Pusa Waxy Maize Hybrid-1	Amylopectin
4.	Pusa Vivek QPM-9 Improved, Pusa HQPM-5 Improved, Pusa HQPM-7 Improved, Pusa HQPM-1 Improved, Pusa Biofortified Maize Hybrid-1, Pusa Biofortified Maize Hybrid-2, Pusa Biofortified Maize Hybrid-3, Pusa Biofortified Maize Hybrid-4, Pusa HQPM-4 Improved & Pusa Super Sweet Corn-3	QPM + PVA
5.	Pusa Biofortified Maize Hybrid-7 & Pusa Biofortified Maize Hybrid-8	QPM + LP
6.	Pusa Super Sweet Corn-4 (APTSKH-1)*	PVA + VitE
7.	Pusa Waxy Maize Hybrid-2 (AQWH-5)*	QPM + Amylopectin
8.	Pusa Biofortified Maize Hybrid-5 & Pusa Biofortified Maize Hybrid-6	QPM + PVA + VitE
9.	Pusa Waxy Maize Hybrid-3 (APQWH-8)*	Amylopectin + QPM + PVA

*Identified for release by AICRP during AICRP Workshop on Maize (2025), QPM: lysine and tryptophan, PVA: provitamin-A, VitE: vitamin-E, LP: low phytate

High amylopectin

Maize grains rich in amylopectin, also called waxy maize, are an important source of diet in North-Eastern states of India and Southeast Asian countries [40, 41]. Waxy maize possesses higher amylopectin (~95-100%) as against normal maize (~70-75% amylopectin) [42, 43]. Fresh waxy cobs are popular as a breakfast food around the world. Waxy maize, due to its higher amylopectin level, is easily digested in the human gut [44, 45]. In professional athletes, waxy maize is widely consumed as a rapid source of energy [46]. The recessive *waxy1* (*wx1*) gene present on chromosome-9 was introgressed into the parents of several elite hybrids. IARI released the country's first waxy hybrid, 'Pusa Waxy Maize Hybrid-1', in 2025 (Table 1).

Multinutrient-rich maize

Several hybrids with a combination of nutritional quality traits developed by MAS have been released by IARI [32, 47]. (Table 1). 'Pusa Vivek QPM-9' (2017) earns the distinction of being the world's first provitamin-A-rich QPM maize cultivar. Similarly, 'Pusa HQPM-5 Improved' (2020), 'Pusa HQPM-7 Improved' (2020), 'Pusa HQPM-1 Improved' (2021), 'Pusa Biofortified Maize Hybrid-1' (2021), 'Pusa Biofortified Maize Hybrid-2' (2022), 'Pusa Biofortified Maize Hybrid-3' (2022), 'Pusa Biofortified Maize Hybrid-4' (2024) and 'Pusa HQPM-4 Improved' (2025) were also rich in protein quality (lysine and tryptophan) and provitamin-A, and released for commercial cultivation in India. 'Pusa Biofortified Maize Hybrid-5' (2025) and 'Pusa Biofortified Maize Hybrid-6' (2025), rich in protein quality, provitamin-A, and vitamin-

E have been released in India. Further, 'Pusa Biofortified Maize Hybrid-7' (2025) and 'Pusa Biofortified Maize Hybrid-8' (2025) with high lysine and tryptophan and low phytate have been developed and identified for release.

Research on novel nutritional traits

Traditional maize possesses up to 30% of amylose, but certain maize genotypes possess >50% of amylose. Recessive *amylose extender1* (*ae1*), located on chromosome-5 is regarded as the key gene that doubles the amylose content in maize [48]. Glycaemic index of traditional maize is ~80, while maize with the *ae1* gene possesses a GI of <50, thus suitable for management of diabetes in humans [49]. Recently, research efforts at IARI, New Delhi, have led to the development of high amylose maize inbreds and hybrids [50]. Further, modern maize cultivars possess 3-4% of oil, whereas high oil corn contains >6% of oil [51]. Researchers at IARI, New Delhi, have introgressed the mutant allele of the *dgat1-2* gene (chromosome-6) and observed 35.7% and 51.6% enhancement of oil and oleic acid, respectively, in improved maize hybrids [52]. Further, the mutant allele of the *fatb* gene (chromosome-9) led to the 36.3% reduction of saturated palmitic acid, thereby enhancing the quality of oil in maize grains. These newly developed hybrids with *dgat1-2* and *fatb* also possess high provitamin-A and vitamin-E, and thereby enhance the bioavailability of these fat-soluble vitamins. In addition, anthocyanins serve as antioxidants and help in preventing various health disorders [53]. In maize, *A1* (chromosome-3), *A2* (chromosome-5), *C1* (chromosome-9), *C2* (chromosome-4), *R1* (chromosome-10), *Pr1*

(chromosome-5), *Bz1* (chromosome-9), and *Bz2* (chromosome-1) are required to produce anthocyanins in maize kernels [14]. Breeders at IARI, New Delhi, have developed anthocyanin-rich maize with high lysine, tryptophan, provitamin-A, and vitamin-E.

Seed production system in India

Availability of quality seed of maize hybrids at an affordable cost to the farmers is crucial for achieving national food security [54]. With an advanced system of variety development, release, and a wide network of seed production and certification agencies, the country has tremendous potential to fulfil its growing demand for quality seed and emerge as a major player in the global seed market [55]. To derive the full benefits of the cultivars, seeds are required in large quantities and made available to farmers for crop production through a generation system of seed multiplication. At the time of release of a hybrid, a small quantity of seed, normally known as 'nucleus seed' of parental lines, is available to the plant breeder. The Indian Minimum Seed Certification Standards (1988), recognizes three generations viz., 'breeder seed' (BS), 'foundation seed' (FS) and 'certified seed' (CS) [BS-FS-CS], which may be increased to maximum five generations viz., BS-FSI-FSII-CSI-CSII in the event of increase in demand for foundation or certified seed [56]. Breeder seed is produced from 'nucleus seed' under the supervision of a qualified plant breeder of a Research Institute/ Agricultural University/ ICAR Institute. This is meant for an initial and recurring increase of foundation seed. Breeder seed production is supervised by a monitoring team of scientists. FS is the progeny of breeder seed and is produced by State Agricultural Universities, National Seeds Corporation, and State Seed Corporation under the technical control of qualified plant breeders or technical officers. Certified seed is produced from foundation seed, and its production is supervised and approved by the certification agency. The seed of this class is produced by the State or National Seeds Corporation and progressive farmers. This seed is the commercial seed, which is available to the farmers for growing. Each time a cultivar goes through the cycle of multiplication, there is a chance of its deterioration through mechanical admixture in addition to mutation and pollination by alien pollen [57]. Thus, strict monitoring of the genetic purity of breeder, foundation, and certified seed is necessary. In case of maize, seeds of parental inbreds are increased under the breeder and foundation seed production programme, while hybrid seed is

produced under the certified seed production programme.

Production of seeds of parental lines

Maize is a cross-pollinated crop owing to its monoecious and protandrous nature [58]. Pollen grains of maize are light in weight and move through the air, thus called anemophilous. Each anther contains about 2000-7500 pollen grains, depending upon genotypes, and approximately 14×10^6 pollen grains could be produced by each tassel [59]. While each of the cobs can produce 200-500 ovules, each lead to the development of an equal number of fused styles and stigma, popularly called silk. Traditionally, homozygous inbreds are derived through 7-8 cycles of rigorous selfing and /or sib mating from heterogenous or heterozygous germplasms. However, with the advent of doubled haploid (DH) technology, homozygous inbreds are produced in 1-2 seasons [60, 61]. Maternal haploid inducer (HI) lines having *MTL* (chromosome-1) and *DMP* (chromosome-9) genes induce 8-10% haploids when crossed as male lines [62]. These HI lines also possess *R1-nj* allele (chromosome-10) for selection of haploid seeds based on anthocyanin pigmentation [63]. A crossed seed with a colourless embryo and coloured endosperm is designated as a haploid seed, while colour in both embryo and endosperm is treated as a true diploid crossed seed [64]. Seeds with no colour in both embryo and endosperm are due to unwanted selfing or crossing by contaminating foreign pollen due to a lack of proper care followed during the crossing programme. Since, temperate maternal HI lines do not adapt well in Indian conditions, breeders at IARI, New Delhi have now developed subtropically-adapted maternal HI lines for DH line production [63]. Colchicine treatment to germinating haploid seeds leads to doubling of chromosomes and the development of homozygous DH seeds [65].

Inbreds are generally maintained through self-pollination, sib-pollination or a combination of these. Inbreds can also be maintained by growing in isolation, allowing open-pollination among all the plants of a specific inbred. Spatial separation by 800 m from other maize growing fields or temporal separation by at least 25 days of gap in sowing of the neighbouring maize field also ensures maintenance of the inbreds in a large plot, where hand-pollination of every plant becomes rigorous and cumbersome. A single cross hybrid with two parents needs two isolations each for the maintenance of BS and FS. Many of the recently released biofortified maize hybrids have been included in the seed chain, and regularly receive indent for BS.

Production of hybrids seeds

Production of single cross maize hybrid requires one isolation under the CS production programme. The successful seed production of maize hybrids depends upon various factors including (i) genetic purity of the female and male parent, (ii) ratio of female parent to male parent in the hybrid seed production field, (iii) removal of off-types, diseased and rogues from the seed production field to prevent the contamination, (iv) detasseling of the female parent at appropriate time, (v) synchronisation of silking in seed parent and the pollen shedding in male parent, and (vi) separate harvesting from the female and male parent rows to avoid mechanical mixture. The male parent should be taller than the female parent for easy dispersal and movement of pollen. Besides, male line should possess tassels with lax type, with a long main branch with many secondary branches producing abundant pollen for a longer period of time. Female parent should bear a productive cob with high number of seeds, besides having complete exertion of the silk in the cob for receiving the pollen. Both male and female parents should have a shorter anthesis-silking interval (ASI) preferably 2-4 days. Planting patterns for hybrid seed production depend principally on the pollen-shedding ability of the male parent, which determines the amount of pollen that is available and nicking or synchronization of flowering of the male and female parents. Commonly followed planting ratios of female and male parents are 4:1, 2:1; 4:2, 3:1 and 6:1 [66, 67].

Achieving synchronization of flowering among parental lines, also known as nicking, is essential for enhancing hybrid seed yield. In general, the female line should flower 1-2 days earlier than the male parent for higher seed setting. Receptivity of silks in females remains for longer days (5-7 days), while pollen availability remains for shorter duration (3-5 days). In the event of non-synchrony, the most common technique for achieving synchronization is staggered sowing of male and female parents. Late-flowering lines are planted earlier than the early-flowering lines. Further, as a precautionary measure, male parents may be planted on two or more dates to extend the duration of pollen availability. Flowering of the late parent can also be manipulated by fertilizer management or agronomical practices (clipping leaves and shaking plants). Non-synchronization of flowering up to 6-8 days can be adjusted by soil application of fertilizer (urea and DAP) and/ or micronutrients, foliar application of urea (1-2%) or growth regulator (GA_3 / NAA/ IAA) at 2-3 days interval at flowering initiation stage in the late

parent to prepone flowering during hybrid seed production [68]. Application of NAA@ 500 ppm thrice at two days intervals before anthesis in late parent reduces the duration of non-synchronisation of flowering in the parental lines of sweet corn. In maize, detasseling is commonly used for hybrid seed production. Detasseling involves physically removing tassels from the female parent before the tassels shed pollen on the female parent. Detasseling is initiated when the tassels emerge from the leaf sheaths before pollen shedding.

Seed certification

Genetic purity in commercial seed production is often maintained through a system of seed certification. This programme is carried out by State Seed Certification Agencies in most of the states of the country. The principal objective of seed certification is to maintain and make available genetically pure seeds. During hybrid seed production, four field inspections viz., one before flowering, two during flowering and one post-flowering are generally made to check the genetic purity of the genotypes. As of now, specifications in place for traditional maize hybrid are followed for biofortified hybrids.

Seed production in farmers' field

Farmers' Participatory Seed Production (FPSP) programme provides great benefits to the farming system through production and distribution of seeds [55]. IARI has been successfully implementing FPSP programme in the vicinity of its main campus in New Delhi, and its regional stations for nearly 20 years. Under the FPSP program, specific crop cultivars which are widely grown and have high demand for cultivation, are given priority. Breeder seed is provided to the identified farmers on cost basis as fixed by the Department of Agriculture and Farmers Welfare (DAFW), Government of India. All information on packages of practices of the particular crop cultivars is also provided in local language. A multidisciplinary team consisting of a breeder of the specific crop, pathologist, entomologist and seed production specialists make visits to the fields of seed growers at regular intervals [69]. Seeds of biofortified maize hybrids such as 'Pusa HQPM-1 Improved' and 'Pusa HQPM-5 Improved' have been produced at IARI under the FPSP programme.

Seed production through commercialization

A large quantity of seeds of biofortified maize hybrids have been produced through commercialization [55]. The Memorandum of Understanding (MoU) signed between

IARI and industry partners, such as private seed companies and Farmer Producer Organizations (FPOs) is valid for five years. Breeder seed is provided to the agro-industries, who in turn have the license for foundation and certified seed production, and marketing, while keeping the name of the cultivar same as released and notified by the Gazette of India. Both 'license fees' and 'royalty at source' are charged during the process of 'non-exclusive' licensing of IARI technologies. Signing of >50 MoUs for biofortified maize hybrids such as 'Pusa HQPM-1 Improved', 'Pusa HQPM-5 Improved', 'Pusa Biofortified Maize Hybrid-1', 'Pusa Biofortified Maize Hybrid-2', and 'Pusa Biofortified Maize Hybrid-3' to >20 agro-industry partners is a testimony to the popularity of the IARI-bred biofortified maize hybrids. Commercialization not only provides a robust foundation to the seed companies and FPOs, especially the small and medium ones with limited or no capacity for their own R&D, but has also led to the large-scale seed production and dissemination among farmers throughout the country. The licencees also included some of the prominent national and Multi-National Companies (MNCs), thereby suggesting the increasing demand of popularity of biofortified maize hybrids in India.

Future prospects

With the increase in cost of detasseling due to the involvement of labour, cytoplasmic-genic male sterility (CGMS) is fast becoming popular in the development of maize hybrids [70]. In maize, three types of cytoplasmic male sterility (CMS) systems viz., CMS-T (Texas), CMS-C (Charrua) and CMS-S (USDA) are available [71]. The male sterility is due to genes present in mitochondria, and they cause disruption in the functional pollen production in maize [72]. The chimeric *T-urf13* gene is responsible for CMS-T [73]. while the chimeric *atp6-C* gene causes male sterility in CMS-C [74]. In case of CMS-S, a repeated DNA region 'R' containing two orfs (*orf355/orf77*) is attributed to the male sterility [75]. Currently, both T and C cytoplasm have been used in the development of male sterile baby corn hybrid development in India. However, to utilize the CMS systems in biofortified maize, the male inbreds should also possess the fertility restorer genes. Presence of both *Rf1* and *Rf2* on chromosome-3 and chromosome-9, respectively, are required to restore fertility in CMS-T [71]. While *Rf3* (chromosome-2) is essential for restoring fertility in CMS-S [76]. While male fertility in CMS-C is fully restored by the *Rf4* gene (chromosome-8) or *Rf5*

(chromosome-8). *Rf1*, *Rf2*, *Rf4*, and *Rf5* possess sporophytic control, whereas *Rf3* behaves through gametophytic control. Thus, the conversion of fertile female inbreds into male sterile versions needs to be initiated at a large scale to convert available fertile hybrids into the CGMS system. Of these, CMS-T and CMS-C provide complete male sterility, while the use of CMS-S showed incomplete sterility due to the influence of environmental factors [77, 78]. It is well known that CMS-T, present in nearly all hybrids in the US, became susceptible to the T-race of Southern Corn Blight (SCB) or Maydis Leaf Blight (MLB) caused by *Bipolaris maydis* during the 1960s, thereby prompting a shift from the CGMS system to manual detasseling. T-race has not been found in India, while the predominant race has been the O-race [79]. Thus, CMS-T can be safely used in India; however, deployment of both T- and C-CMS is desirable, as in the event of any breakout of a specific race of SCB, alternate CMS can be effectively used for commercial cultivation.

Conversion of male fertile inbreds into the male sterile version is a time-consuming process, as 6-7 generations of backcrosses with the male sterile source are required. Kermicle [80]. reported a 3% induction of androgenic or paternal haploids caused by the mutation in *indeterminate gametophyte1 (ig1)* (chromosome-3) in the temperate maize inbred, Wisconsin-23 (W23). Paternal haploid inducer (HI) line, as opposed to the maternal HI line, is employed as the female parent to induce the haploids of the source germplasm. As a result, the induced haploids contain the cytoplasm from the inducer line and the nuclear genome from the source germplasm [81]. Such exchange of cytoplasm indicates its potential use in rapid conversion of inbreds to their cytoplasmic male sterile (CMS) form [81]. The wild-type *ig1* codes for a transcription factor with a Lateral Organ Boundaries (LOB) domain (LBD) that governs lateral organ development and is reported to regulate the expression of proliferation-promoting genes in the embryo sac [82]. The mutant *ig1*, having a *Hopscotch* transposable element, has been introgressed into a subtropical genetic background, and locally adapted paternal HI lines are now available with IARI. These newly developed HI lines with specific male sterile cytoplasm can be crossed as female with the fertile line that needs to be converted to male sterile version. The haploid plants with cytoplasm coming from female line and haploid nuclear genome donated by male line, can be rapidly converted to doubled

haploid (DH) line when pollinated with the fertile male line [83].

Further, the majority of traits targeted by nutritional quality enhancement are recessive in nature [1, 20]. Thus, unwanted pollination by the pollen carrying the dominant/wild-type allele from the neighbouring field dilutes the nutritional quality. Unilateral cross-incompatibility (UCI) that prevents pollen from a foreign parent from successfully fertilizing an egg can be effectively used to prevent such cross-pollination. Maize has nine known cross-incompatibility systems located throughout the genome: *gametophyte factor 1 (ga1)*, *ga2*, *ga3*, *ga4*, *ga6*, *ga7*, *ga8*, *ga10*, and *teosinte crossing barrier 1 (tcb1)* [84, 85]. Of these, *Tcb1* is predominantly present in teosinte, and *Ga1* and *Ga2* found in both maize, especially popcorn accessions, and teosinte, provide significant promise in their application [86]. *Ga1*, located on chromosome-4 is composed of three different alleles, *Ga1s*, *Ga1m* and *ga1*. Generally, popcorn genotypes possess the *Ga1s* haplotype, and dent corn possesses the *ga1* haplotype [87]. Plants with *ga1* accept any type of maize pollen; *Ga1s* plants cannot be pollinated by *ga1* pollen, but can pollinate all other genotypes; while *Ga1m* plants exhibit wide compatibility in either corn types. Further, *Ga2* has been mapped on chromosome 5 [88]. *Ga2* locus also possesses three alleles viz. *Ga2s*, *Ga2m*, and *ga2* [89]. While *Tcb1* located on chromosome-4 was first observed in *Z. mays ssp. mexicana* collected from Central and Southern Mexico [90]. Three alleles, such as *Tcb1s*, *Tcb1m* and *tcb1* have been reported so far in maize germplasm pool [91, 92]. The mechanism of pollen rejection in *Ga2* and *Tcb1* remains the same as *Ga1*. Thus, introgression of *Ga1s*, *Ga2s* and *Tcb1s* genes and their stacking would help in rejecting *ga1*, *ga2*, and *tcb1* alleles, respectively present in contaminating foreign pollen [93]. This would ensure the genetic purity of nutritional quality of maize grains in farmers' fields.

CONCLUSIONS

Intensive research efforts in India have led to the development of a large number of biofortified maize hybrids in India. Introgression of genes such as *o2*, *crtRB1*, *lcyE*, *vte4* and *wx1* has increased the nutritional qualities such as protein quality (lysine and tryptophan), provitamin-A, vitamin-E and amylopectin, while *lpa1* and *lpa2* genes reduced the level of phytate in maize grains. Stacking of multiple traits helped in combining several nutritional traits in a single genetic background. Availability of molecular markers associated with the target genes

helped in accelerating the breeding cycle and development of hybrids. The traditional seed production system, coupled with the FPSP programme and commercialization, has generated a large quantity of seeds. Integration of CGMS and UCI systems in biofortified maize would further help in their popularization.

CONFLICT OF INTEREST

It is to be certified that there is no conflict of interest among the authors/institutes.

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