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INVITED ARTICLE
Nutritional Enrichment of Maize through Molecular Breeding

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ABSTRACT

Malnutrition affects a large proportion of population, and has emerged as a serious health issue worldwide. Besides affecting growth and development in humans, malnutrition also contributes to poor socio-economic development. Maize is one of the most important cereal crops, and used as an important source of food and feed, thereby provides valuable source energy. However, traditional maize is poor in nutritional qualities. Essential amino acids like lysine and tryptophan, vitamins such as vitamin-A and vitamin-E, and minerals like iron (Fe) and zinc (Zn) are present in low concentration in maize kernels. Recessive genes like *opaque2* and *opaque16* enhance lysine and tryptophan, while natural variants of *crtRB1* and *lcyE* increase the concentration of vitamin-A in maize kernel. In addition, mutant *vte4* gene causes enhancement in vitamin-E, while mutated versions of *lpa1* and *lpa2* reduce phytic acid thereby enhance the bioavailability of Fe and Zn in the maize grains. Availability of molecular markers provide opportunity to undertake molecular breeding for accelerating the breeding cycle and development of biofortified maize hybrids. Here, we presented the status of prospects of development of biofortified maize hybrids through molecular breeding with a special reference to India. We also presented various challenges and opportunities to popularize the newly developed nutritionally enriched maize hybrids.

Keywords: *Biofortification, Maize, minerals, molecular marker , protein and vitamins.*

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Dr. Firoz Hossain *is a Principal Scientist at the Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi. He is currently the Programme Leader of Maize Breeding Programme at IARI. He has >20 years of experience in working with maize. His area of interest includes improvement of nutritional quality and specialty traits in maize. Dr. Firoz and his team has released 11 biofortified maize hybrids developed through molecular breeding. 'Pusa Vivek QPM9 Improved' developed by his team earns the distinction of being world's first provitamin-A rich QPM hybrid. He has also developed maize hybrids improved for vitamin-E as well. He has led several externally projects funded by ICAR and Department of Biotechnology. Dr. Firoz is a Fellow of National Academy of Agricultural Sciences (NAAS), New Delhi. He has published >130 research articles in journals of repute. He is the recipient of various awards. He is also involved in teaching Genetics to the Post Graduate students. He has guided five M.Sc. and six PhD students. Several students and Post Docs have been trained in the field of molecular breeding of maize in his lab*

Malnutrition caused due to consumption of unbalanced food affects people throughout the globe (Duo *et al*., 2024). It leads to increased morbidity, disability, abnormal physical and mental health, and contributes to poor socio-economic development worldwide (Hossain *et al*., 2023). Continued intake of poor quality diet can lead to different forms of malnutrition, including under-nutrition, micronutrient deficiencies, overweight and obesity (WHO, 2023; Neeraja *et al*., 2022). Nearly, 3.1 billion global populations can not afford healthy food, of which 735 million people are hungry (FAO, IFAD, UNICEF, WFP and WHO, 2023). Globally, 148.1 million (22.3%) children (<5 years) are stunted, while 45 million (6.8%) children possess wasting and 37 million (5.6%) children are affected by obesity. Under nutrition causes ~45% death among children (<5 years) mainly in low and middle-income countries. Malnutrition is so widespread that 88% of the countries experience a high level of at least two types of malnutrition, while 29% experience three types of malnutrition (Global Nutrition Report, 2018). Considering the paramount importance of alleviating malnutrition, world leaders at United Nations framed 'Sustainable Development Goals' (SDGs) for meeting the current needs without affecting future generations (UNDESA, 2023). Of the 17, 12 goals are highly associated with nutrition. Alleviating malnutrition is the most cost-effective step as every $1 invested in proven nutrition programme offers benefits worth $16 (Global Food Policy Report, 2016). Thus, balanced and nutritious diet for people assumes great significance to mitigate malnutrition (Gupta *et al*., 2015). Various approaches used to ensure nutrition are food-fortification, medical-supplementation, and dietary-diversification to alleviate the micronutrient malnutrition. However, these avenues are not sustainable in the long run, and their implementation is often limited by lack of purchasing power, poor infrastructure, crop seasonality, expense, and lower bioavailability (vaan Lieshout and de Pee 2005; Bouis and Welch, 2010). 'Crop biofortification' – a process of increasing nutrient density in edible seeds through breeding, has emerged as the most promising approach. Compared to other approaches, biofortification offers several advantages viz., (i) most sustainable (ii) cost-effective, and (iii) provides nutrients in natural form to alleviate malnutrition (Pfeiffer and McClafferty, 2007; Yadava *et al*., 2018).

Maize has emerged as the most important cereal crop of the globe (Erenstein *et al*., 2022; Gao *et al*., 2024). Apart from food and feed usage, maize also provides raw materials to corn syrup, emulsifier, textile-, paper- and adhesive- industries (Talukder *et al*., 2023). It is grown across North America and South America, Africa, Asia and Europe that touched production of 1163.49 mt from 203.47 mha area and productivity of 5.71 t/ha (FAOSTAT, 2022). In India as well, maize is an important crop with a production of 38.08 mt from an area of 10.74 mha with a productivity of 3.55 t/ha during 2022-23 (www.upaj.gov.in). In India, maize is used by the poultry sector (47%), livestock (13%), food (13%), starch industries (14%), processed food (7%), exports and others (6%) (Rakshit *et al*., 2019). However, traditional maize grain is poor in nutritional qualities such as protein quality (lysine and tryptophan), provitamin-A, vitamin-E, iron (Fe) and zinc (Zn) (Neeraja *et al*., 2022). Availability of molecular markers associated with the target genes for nutritional quality provides great opportunity to accelerate the breeding cycle by involving nearly the half of the time required in conventional breeding (Table 1). Besides, (i) the selection of the target gene is more precise, (ii) the lengthy progeny testing can be avoided, (iii) desirable plants can be selected at the seedling stage thereby saves resources, and (iv) the high cost involved in phenotyping of the segregating populations is also reduced drastically. Here, we present the status of genomics-assisted breeding programmes on maize biofortification in India.

Lysine and tryptophan

Traditional maize endosperm protein is deficient in lysine and tryptophan, which is less than half of the recommended dose specified for human nutrition (Vasal, 2001). Lysine and tryptophan serve as precursors for several neuro-transmitters and metabolic regulators, and their deficiency leads to reduced appetite, delayed growth, impaired skeletal development and aberrant behaviour in humans (Yadava *et al*., 2022). The recessive allele of the *Opaque2* (*O2*) gene located on chromosome-7 causes doubling of lysine and tryptophan. It was first described by Jones and Singleton in early 1920s (Emerson, *et al*., 1935), but its nutritional significance was discovered by Mertz *et al*. (1964) at Purdue university, USA. The α-zeins are the most abundant proteins in the endosperm but characteristically poor in essential amino acids viz., lysine and tryptophan. The homozygous *o*2 mutant causes a decrease of the production of zeins resulting in a corresponding increase in non-zein proteins, which naturally contain higher levels of lysine and tryptophan (Gibbon and Larkins, 2005). The *o2* gene encodes a basic-domain-leucine-zipper (bZIP) transcription factor that regulates the expression of the 22 kDa α-zein and several other genes. Mutation at the *o2* locus encodes the defective regulatory element resulting in reduced transcription of the 19 kDa and 22 kDa α-zein genes. The 19 kDa and 22 kDa α-zeins constitute almost 70% of the total maize endosperm zeins and the reduction in α-zein transcription results in reduction of zein proteins relative to non-zeins. The *o2* gene also regulates the lysine ketoglutarate reductase (LKR) gene (Schmidt *et al*., 1987). The LKR gene encodes the protein that degrades the free lysine. The *o2* mutation produces a defective transcription factor resulting in reduced transcription of the LKR and less degradation of free lysine in a mutant relative to wild-type (Brochetto-Braga *et al*., 1992). In addition, it also balances leucine-isoleucine ratio for tryptophan liberation which enhances niacin biosynthesis and combats pellagra disease.

Lysine and tryptophan levels in maize inbreds adapted to India have been increased through introgression of *o2* allele into several normal maize inbreds through conventional as well as molecular breeding (Gupta *et al*., 2013). These *o2*-based simple sequence repeats (SSR) markers (*phi057*, *phi112* and *umc1066*) have been used to convert non-QPM lines into their QPM versions (Hossain *et al*., 2018). ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan (VPKAS), Almora, developed 'Vivek QPM9' – a QPM hybrid developed through marker-assisted selection (MAS), and was released during 2008 (Table 2). In 2017, three QPM hybrids viz., 'Pusa HM4 Improved', 'Pusa HM8 Improved' and 'Pusa HM9 Improved' were developed by ICAR-Indian Agricultural Research Institute (IARI), New Delhi (Hossain *et al*., 2018). Besides, 'VL QPM Hybrid-45' was released by VPKAS, Almora during 2022, while 'QPMH-6' developed by ICAR-Indian Institute of Maize Research, Ludhiana was released for commercial cultivation in 2024 (Table 2).

Further, a recessive *opaque16* (*o16*) gene present on chromosome-8 has also been found to increase nutritional value in maize (Yang *et al*., 2005; Sarika *et al*., 2017). Mutant combination of *o2* and *o16* offers possibility of enhancement of lysine by 40-80% over *o2* genotype alone. Working with *o16*, we could conclude that it was as good as *o2* mutant in terms of lysine and tryptophan level, but did not induce opaqueness –a great advantage to the breeders (Sarika *et al*., 2018a). We have also pyramided *o2* and *o16* in the genetic background of four QPM hybrids, and found that *o2o2/o16o16*-based hybrids possessed an average enhancement of 49% and 60% in lysine and tryptophan over the original hybrids, with highest enhancement amounting 64% and 86%, respectively (Sarika *et al*., 2018b). Chand *et al*. (2022) also observed that *o2o2/o16o16*-based hybrids also possessed 51% and 43% higher lysine and tryptophan, respectively over *o2*-based checks.

Provitamin-A

Vitamin-A is essential for normal functioning such as proper visibility, maintenance of cell function, epithelial integrity, red blood cell production, immunity and reproductive systems in humans (Sommer and West, 1996). Vitamin-A deficiency (VAD) affects about 4.4 million preschool-age children and 20 million pregnant women across the world (www.harvestplus.org). Though yellow maize possesses tremendous natural variation for carotenoids, it is predominated by lutein and zeaxanthin (Vignesh *et al*., 2012, 2013; Muthusamy *et al*., 2015a, b, c, 2016). Provitamin-A carotenoids are present <2 ppm as compared to target level of 15 ppm (Pixley *et al*., 2013).

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. Naturally available *lcyE* gene converts lycopene into ζ-carotene and eventually to α-carotene through the action of other associated genes. Favourable *lcyE* allele forces pathway flux towards β-carotene branch (Harjes *et al*., 2008). Though the favourable *lcyE* allele increases the proportion of β-carotene in the pathway, a large amount is hydroxylated to produce β-cryptoxanthin (with 50% provitamin A activity) and zeaxanthin (0% provitamin A activity). *crtRB1* is a hydroxylase gene that converts β-carotene into β-cryptoxanthin. However, naturally available favourable *crtRB1* allele blocks the process of hydroxylation of β-carotene in to further components, thereby increases the concentration of β-carotene in the kernel (Yan *et al*., 2010).

Due to development and access to reliable PCR-based gene specific markers for *lcyE* and *crtRB1* genes, MAS has become an attractive option for provitamin-A enrichment in maize (Harjes *et al*., 2008; Yan *et al*. 2010; Babu *et al*. 2013; Zunjare *et* *al.* 2017, 2018a,b,c). Quantifying the provitamin-A carotenoids of maize samples using high performance liquid chromatography (HPLC) is difficult, time-consuming and expensive, and breeding programme thus would benefit greatly from use of MAS to reduce the need for phenotypic assays. By selecting for the favourable alleles of the two key genes viz., *lcyE* and *crtRB1*, provitamin-A concentration can be increased in the maize endosperm (Babu *et al.*, 2013). Breeders at IARI, New Delhi, have introgressed the favourable allele of *crtRB1* gene into parents of elite hybrids adapted to diverse ecological regions of India. The *crtRB1*-derived hybrids showed that kernel β-carotene is as high as 21.7 ppm, compared to 2.6 ppm in the original hybrids (Muthusamy *et al.*, 2014). This is the *'first-ever demonstration of conversion of elite maize hybrids into β-carotene-rich version'*. Later, four QPM hybrids were also introgressed with *crtRB1*- and *lcyE*-favourable alleles for elevation of provitamin-A (Zunjare *et al.*, 2018a). The introgressed hybrids showed a mean of 4.5-fold increase in provitamin-A (range of 9.25-12.88 ppm), compared to original hybrids (2.14-2.48 ppm). Provitamin-A rich maize hybrids released in India included 'Pusa Vivek Hybrid-27 Improved' during 2020, and 'VL Vita' during 2024 by IARI, New Delhi and VPKAS, Almora, respectively (Table 2).

Vitamin-E

Vitamin-E or tocopherol is an essential micronutrient for reproduction and quenches free radicals in cell membrane. It protects the humans from cardiovascular disease, Alzheimer disease, neurological disorder and many age-related degenerations. Maize kernels are rich in total tocopherol of which, γ-tocopherol constitutes ~80% and α-tocopherol accounts ~20% of the total pool. Due to favourable interaction with the receptor, α-tocopherol is present 10 times more than γ-tocopherol in plasma of humans. It is estimated that over 20% of the examined people both in developed and developing countries has suboptimal plasma α-tocopherol. A favourable allele of *vte4* (chromosome-5)*, coding γ-tocopherol methyl transferase* (γ-TMT) accumulates α-tocopherol by 3.2-fold (Li *et al.*, 2012). Li *et al.* (2012) has reported two insertion/ deletions (*InDel7* and *InDel118*) within the gene *vte4* involved in tocopherol biosynthesis pathway and an SNP at 85 kb upstream of *vte4* thereby significantly affecting level of α-tocopherol. *InDel118*, located 9-bp upstream of the putative transcription start site, controls α-tocopherol by regulating *vte4* transcript level, whereas *InDel7* by effecting translation efficiency (Li *et al.*, 2012). Gene-based markers specific to *vte4* provided opportunity to undertake molecular breeding in maize.

In India, an effort to enhance vitamin-E level in maize was undertaken at IARI, New Delhi. Four Indian elite QPM and provitamin-A rich inbreds were targeted for enhanced vitamin-E to develop multi-nutrient maize. One of the exotic inbreds with favourable allele for both the *InDels* and higher α-tocopherol, was used as donor for enhancement of α-tocopherol. Introgression of favourable alleles of *vte4* from donor to the recipient was successfully carried out by MAS (Das *et al.*, 2018). The *vte4*-based reconstituted hybrids showed a 2-fold enhancement in α-tocopherol (16.83 ppm) over original hybrids (8.06 ppm) (Das *et al.*, 2021). 'APTQH-5' rich in α-tocopherol has been identified for release, and it is expected to be released for commercial cultivation in 2024.

Iron and zinc

Humans require iron (Fe) for basic cellular functions and proper functioning of the muscle, brain and red blood cells (Roeser, 1986). Zinc (Zn) is an essential mineral for humans, animals and plants for many biological functions. It plays a crucial role for more than 300 enzymes in the human body for the synthesis and degradation of carbohydrates, lipids, proteins and nucleic acids (Sandstorm, 1997). Target of 60 ppm of Fe and 38 ppm of Zn (on dry weight basis) has been fixed in maize (Bouis and Welch, 2010). Presence of multiple minor loci, high genotype × environmental interactions and dilution effects in hybrids pose major challenges to breed for high Fe and Zn rich maize. Experimental hybrids with 40 ppm of Fe and 30 ppm of Zn have been identified.

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 the eukaryotic cells. These phosphate groups impart PA a strong negative charge at cellular pH, as a result it tightly binds positively charged monovalent or bivalent mineral cations to form mixed salts referred to as phytate or phytin (O'Dell *et al.*, 1972). Owing to negative charge, PA or $InsP_6$ are potent chelators of nutritionally important positively charged mineral ions viz., Fe and Zn. When consumed, dietary PA and its isomers continue to bind seed derived minerals from food items, making them unavailable for absorption in the gut (Raboy, 2020). 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 types, respectively (Shi *et al.*, 2005). In low PA mutant (*lpa*), total phosphorus content remains the same as in wild type grains, however PA content is greatly reduced coupled with proportional increase in free inorganic P (Raboy *et al.*, 2000). In India, Ragi *et al.* (2021) developed *lpa1-1*-based maize inbreds that possessed 35.8% lower PA (1.68 mg/g) than the wild-type inbreds (2.61 mg/g). Further, Ragi *et al.* (2022) reported that *lpa2-1*-based mutant inbreds possessed significantly low mean PA (1.90 mg/g) over wild-type inbreds (2.56 mg/g) with average reduction of 26% PA among *lpa2-1* mutants. Besides, *lpa2-2* was successfully introgressed into regionally well adapted and productive elite inbred lines through MAS at Tamil Nadu Agricultural University (TNAU), Coimbatore (Sureshkumar *et al.*, 2014; Tamilkumar *et al.*, 2014), respectively. These lines can be used for the development of hybrids with low PA. Yathish *et al.* (2022) introgressed *lpa2-2* gene into elite inbreds, and the introgressed progenies possessed low PA (2.37-2.40 mg/g in improved inbreds) compared to 3.16-3.59 mg/g in recurrent parents, thereby reducing the phytate by an average of 24-34%. 'PMH-1-LP' developed by IIMR, Ludhiana is a low phytate maize hybrid released in India during 2022 (Table 2).

Multinutrient-rich maize

Several hybrids with combination of nutritional quality traits have been developed and released. 'Pusa Vivek QPM-9' (2017) earns the distinction of being 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) and 'Pusa Biofortified Maize Hybrid-3' (2022) were also rich in protein quality (lysine and tryptophan) and provitamin-A, and released for commercial cultivation in India (Table 2). APTQH-5 rich in protein quality, provitamin-A and vitamin-E have been identified for release in India. These hybrids have been developed through molecular breeding approaches.

Challenges and opportunities

Despite great health benefits, QPM cultivars account for only 1% or less of 90 million hectares grown in Mexico, Latin America, Sub-Saharan Africa and Asia (CIMMYT, 2012). India is also not an exception in this regard despite the availability of diverse biofortified hybrids. Successful adoption of biofortified maize cultivars depends on various factors related to research and development, socio-economic issues and policy interventions (Gupta *et al.*, 2015). Some of the factors that warrant urgent attention is mentioned below.

It is perceived that nutritionally enriched crops possess low yielding potential. QPM, provitamin-A, vitamin-E, Fe and Zn do not have any yield penalty, and nutritionally enriched maize for these traits can provide grain yield similar to normal maize (Gupta *et al.*, 2015). MAS-derived QPM, provitamin-A, vitamin-E and low phytate version of hybrids have been tested under the AICRP trials, and were found to be *at par* with the original versions for grain yield potential (Muthusamy *et al.* 2014; Gupta *et al.*, 2015; Hossain *et al.*, 2018). Germplasm base of nutritionally enriched maize is quite narrow, primarily due to the fact that very few breeding centres have active quality breeding (Hossain *et al.*, 2016). Therefore, strengthening of research collaborations among various national partners of the National Agricultural Research System (NARS) and international research organizations like CIMMYT and HarvestPlus would help in sharing novel germplasm and expertise for the development of biofortified maize. Establishment of 'nutritional quality service labs' are essential for assessing large number of segregating progenies in the breeding programme. Creation of trained human resources for precise estimation of the nutritional quality is also key to the success. Further, the effects of micronutrients are invisible, and farmers would face difficulty in convincing the trader regarding the extent of quality of his produce while selling in the market. Hence, development of a portable device that rapidly determines the quality would be of great help to the farmers. Nutrition rich hybrids once pollinated by pollen from normal maize, show xenia effects leading to dilution of quality (Gupta *et al.*, 2015). In case of QPM, xenia effects caused contamination to an extent of 11% of the total harvest (Ahenkora *et al.*, 1999).

Growing of trait specific biofortified variety in larger area would reduce the loss in nutritional quality. Thus, the requirement of separate post-harvest processing and storage arrangements for biofortified maize grains is an essentiality to avoid contamination from normal maize grains.

Biofortified maize with higher micronutrients may find important place among health-conscious urban population, as consumers are ready to pay 20-70% premium price for the biofortified foods (Steur *et al*., 2015). Attractive labelling and suitable branding highlighting the health benefits on products made from biofortified maize would help the consumers to choose more nutritious foods over conventionally available ones. To meet the industrial requirement, biofortified maize grains need to be systematically evaluated, and 'contract farming and buy-back policy' would ensure continuous supply of grains to the industry. In Asian countries including India, 60-70% of the maize is used as animal feed, and biofortified maize is advantageous over normal maize. QPM in poultry diet improves the growth performance of broilers and results in higher weight gains when replaced with normal maize (Panda *et al*., 2013). A study on effect of provitamin-A biofortified maize diet on meat quality in Ovambo chickens has been conducted in Africa (Odunitan-Wayas *et al*., 2016). The results revealed that the provitamin-A fed chickens had higher redness and yellowness and lower lightness in the meat and skin colour than white maize fed chickens. Further, QPM and provitaminA enriched diet improved feed efficiency, reduced abdominal fat and increased breast muscle in chicken during nursery phase over other types of maize-based diet (Prakash *et al*., 2021). Prakash *et al*. (2023) also reported beneficial effect of low phyate maize on bone breaking strength and intestinal phosphorus transporters in slow growing chickens during nursery phase. Panda *et al*. (2012) further studied the utilization of QPM in the diet of layers (28-44 weeks), and found that replacement of normal maize with QPM increased egg production and improved feed efficiency. Sensitization of poultry growers on other benefits of biofortified maize on poultry industry would further help in its popularization.

Altered phenotypes caused due to introgression of new trait may prove to be a deterrent to the easy acceptability of biofortified grains among consumers. In specific areas including India, white maize is still preferred, and provitamin-A rich maize having orange/yellow colour may not be easily accepted (De Groote *et al*., 2010). Strong extension activities may play a major role in the popularization of biofortified crops. Further, specific training of extension workers and volunteers, arrangement of community drama, radio broadcasts, and other activities such as field days, training for grandmothers and community leaders, and market promotion events would help in the promotion of biofortified maize. Policy supports from the government are essential for the successful adoption of biofortified maize cultivars (Gupta *et al*., 2015). Intensive awareness campaign supported by the government would help in popularization of biofortified maize for its nutritional value. The available biofortified maize can potentially contribute to the nutritional security especially in the North-Eastern states and tribal areas in India. Inclusion of biofortified maize in the government sponsored programmes like National Food Security Mission (NFSM), Rashtriya Krishi Vikas Yojna (RKVY) as well as nutrition intervention programme such as Integrated Child Development Scheme (ICDS) and 'Mid-day meal' scheme would help in further popularization (Yadava *et al*., 2018). Enhanced minimum support price (MSP) should be provided to biofortified grains over traditional maize, as value of the nutritional quality should also be included while calculating MSP. Intervention such as creation of 'Seed village' would strengthen the seed chain to produce and supply good quality seeds of biofortified maize to industry. Providing subsidized seeds and other inputs would further contribute to the rapid dissemination of nutritionally improved cultivars among the farmers.

CONCLUSION

Molecular breeding has led to the accelerated development of newly developed biofortified maize hybrids possess higher protein quality (lysine and tryptophan), vitamins (-A and -E) and bioavailability of minerals (Fe and Zn). Molecular markers have also facilitated stacking of multiple traits in the single genetic background. These biofortified hybrids are also high yielding, thus *at par* with the traditional maize. With proper awareness, quality seed production, effective linkages with industry and strong policy support, these biofortified maize hybrids would play a vital role in

Table 1: Details of nutritional improvement of maize using marker-assisted selection

S. No.	Trait	Genes	Chromosome	Marker type	Reference(s)
1.	Lysine and Tryptophan	*opaque2*	7	Gene-based SSR	Gupta *et al*. (2013)
2.		*opaque16*	8	Linked SSR	Yang *et al*. (2005)
3.	Provitamin-A	*crtRB1*	10	Gene-based *InDel*	Yan *et al*. (2010)
4.		*lcyE*	8	Gene-based *InDel*	Harjes *et al*. (2008)
5.	α-tocopherol	*vte4*	5	Gene-based *InDel*	Li *et al*. (2012)
6.	Low phytate	*lpa1-1*	1	Gene-based SNP	Abhijit *et al*. (2020)
7.		*lpa2-1*	1	Gene-based CAPS	Abhijit *et al*. (2020)
8.		*lpa2-2*	1	Linked SSR	Sureshkumar *et al*. (2014)

Table 2: Details of released biofortified maize hybrids developed through molecular breeding

S. No.	Name of hybrid	Nutritional traits	Year of release	Institutions
1.	Vivek QPM9	Lysine + tryptophan	2008	VPKAS, Almora
2.	Pusa HM4 Improved	Lysine + tryptophan	2017	IARI, New Delhi
3.	Pusa HM8 Improved	Lysine + tryptophan	2017	IARI, New Delhi
4.	Pusa HM9 Improved	Lysine + tryptophan	2017	IARI, New Delhi
5.	Pusa Vivek QPM9 Improved	Lysine + tryptophan + Provitamin-A	2017	IARI, New Delhi
6.	Pusa Vivek Hybrid-27 Improved	Provitamin-A	2020	IARI, New Delhi
7.	Pusa HQPM7 Improved	Lysine + tryptophan + Provitamin-A	2020	IARI, New Delhi
8.	Pusa HQPM5 Improved	Lysine + tryptophan + Provitamin-A	2020	IARI, New Delhi
9.	Pusa HQPM1 Improved	Lysine + tryptophan + Provitamin-A	2020	IARI, New Delhi
10.	Pusa Biofortified Maize Hybrid-1	Lysine + tryptophan + Provitamin-A	2021	IARI, New Delhi
11.	Pusa Biofortified Maize Hybrid-2	Lysine + tryptophan + Provitamin-A	2022	IARI, New Delhi
12.	Pusa Biofortified Maize Hybrid-3	Lysine + tryptophan + Provitamin-A	2022	IARI, New Delhi
13.	PMH-1-LP	Low phytate	2022	IIMR, Ludhiana
14.	VL QPM Hybrid-45	Lysine + tryptophan	2022	VPKAS, Almora
15.	QPMH-6	Lysine + tryptophan	2024	IIMR, Ludhiana
16.	VL Vita	Provitamin-A	2024	VPKAS, Almora

alleviating malnutrition through sustainable and cost effective approach.

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