Nutritional quality improvement in maize (Zea mays): Progress and challenges

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ABSTRACT

Malnutrition is one of the important problems which affect the overall human productivity costing huge economic losses to the nations. Addressing malnutrition problem is one of the important components of sustainable development goals. In this context, biofortification of staple food crops could be one of the most practical, environment friendly, cost effective and sustainable approaches in the long run. Maize (Zea mays L.) being staple food crop for more than 900 million populations across the globe, enhancing the nutrient content along with yield is of paramount importance. Maize display large genetic diversity for all the quality parameters and several mutants are available each of the quality traits. Across the globe, several efforts have been made to identify new gene(s) and QTLs for different quality traits and their mobilization to develop new and improved biofortified cultivars. However, any technology or product remains meaningless unless it reaches the main stakeholders. The main stakeholders are the poorest of poor of the society who are most affected due to malnutrition. In order to make biofortification a success story, there is need to address several challenges like appreciated support price for the produce, dedicated production zones, value addition and supply chain development. The policy intervention with respect to sensitization on importance of nutrient rich cultivars and their acceptance by farmers, traders and consumers are the key to success.

Key words: Genes, Maize, Mutant, Policy, Quality

Maize (Zea mays L.) is the most imperative cereal crop worldwide with the highest global production of 1060 million tonnes (FAOSTAT 2017). It is used as food in humans, feed for poultry and livestock, and raw material for an array of industrial- and processed-products (Yadav et al. 2015). More than 900 million people depend on maize for their staple food around the world particularly in the Latin America, Africa and Asia including India (Shiferaw et al. 2011). Maize provides 62% of the proteins from all cereals in Meso America, while it is 43% in Eastern and Southern Africa, 28% in Andean Region, 22% in West and Central Africa and 4% in South Asia (Hossain et al. 2018).

Malnutrition has emerged as one of the major problems especially in under-developed and developing countries of the world (Bouis and Seltzman 2017). The resource-poor suffers the most from ‘hidden hunger’, a term more often used to describe malnutrition. Approximately two billion people are being short of essential micronutrient like iron and vitamin A in their daily diet at global level (Global Nutrition Report 2017). Nearly 45% of deaths of children under age of five are linked to malnutrition (Black et al. 2013). Malnutrition contributes to global burden of disease, and loss in annual GDP in Asia and Africa to the extent of 11% (IFPRI 2016).

Considering the paramount importance of balanced nutrition, global community has set ‘Sustainable Development Goals’ (SDGs) to chart a path towards meeting current human needs without compromising the ability of future generations to meet their needs. Of the 17 goals, 12 contain indicators that are highly relevant to nutrition, reflecting central role of nutrition in sustainable development. Improved nutrition is the platform for progress in health, education, employment, female empowerment, and poverty elimination. It has been estimated that alleviating malnutrition is one of the most cost-effective steps with every $1 invested in proven nutrition programme offers benefits worth $16 (IFPRI 2016). Thus, efforts directed towards providing the balanced and nutritious food assumes great significance (Zunjare et al. 2018, Sarika et al. 2018).

Agricultural systems have traditionally focussed mostly on increasing productivity. However, now research policies must focus that not only provide enough calories to...
meet the energy needs of the poor, but also deliver all the essential nutrients needed for adequate nutritional health. ‘Biofortification’ a process in which micronutrient density in crops is increased through plant breeding, is proposed as a sustainable and cost-effective mean for providing the required levels of nutrition in natural form to alleviate malnutrition in humans (Gupta et al. 2015). Among various micronutrients, lysine, tryptophan, vitamin A, vitamin E, iron (Fe) and zinc (Zn) have remained deficient in endosperm among traditional maize varieties.

Maize is also considered as a crop of industrial importance due to its wide utilisation in industry as raw material and also its role in the world economy and trade (Yadav et al. 2015). Maize grains are rich source of carbohydrate which possesses diverse usage as an industrial raw material. Sticky maize or high amylopectin maize is a popular choice in South-East Asian countries (Devi et al. 2017). Corn oil is also gaining popularity due to desirable fatty acid composition; rich source of linoleic acid (18:2), oleic acid (18:1), palmitic acid (16:0), and steric acid (18:0), small amounts of linolenic acid (18:3), and trace amount of other fatty acids. Further, as compared to other edible oils, maize oil has the advantage of being low in the proportion of mono-saturated fatty acids (Rakshit et al. 2003). Besides, specialty corns like sweet corn, baby corn and pop corn have become popular choice worldwide (Mehta et al. 2017, Yadav et al. 2015). Thus, development of biofortified maize cultivars has huge potential in alleviating malnutrition problem at global level as huge natural variation in the form of mutants or otherwise, existed in maize for several nutritional traits like provitamin A, vitamin E, high-lysine and –tryptophan etc. In this review, we report availability of different mutants, their effects on target traits, utilization in the breeding programme followed by the challenges for their dissemination.

**Genetic variation for nutrient content in maize**

The carbohydrates, proteins, fats, vitamins, minerals, fiber and water are the main nutrients required to fulfill daily needs of human body (Welch and Graham 2004). Considerable variations are reported in maize germplasm for different nutrients. These nutritional components are essential for growth, development, immunity, reproduction, metabolism and other physiological functions.

The important source of energy which converts the glucose into energy is starch, which varies from 59.60 - 74.40% in maize kernel (Cook et al. 2012, Guo et al. 2013, and Yangchong et al. 2013). The kernel energy density is about 365 kcal/100 g, which is close to rice (360 kcal/100g) and wheat (340 kcal/100g) (USDA Natl. Nutrient Database, https://ndb. nal. usda. gov/ndb/). Assuming 90% energy availability, an average male requires a daily energy requirement of 2800 kcal (Mertz 1970, Brown et al. 1988). That means if total energy requirement is to be met from maize nearly 600 g is needed per day. The starch is composed of amylose and amylopectin and the variations for amylose and amylopectin have been reported upto a maximum of about 80 and 100%, respectively (Hallauer 2000). Considerable variations are observed in maize germplasm for different nutrients (Table 1). The protein which plays a major role in enzymatic and hormonal activities, content in maize kernel ranges from 4.50-13.24% (Enyisi et al. 2014, Ai and Jane 2016, Pedersen et al. 2014, Cong et al. 2015, Butts-Wilmmsmeyer et al. 2017). Essential amino acid lysine required for proper growth and muscles development range from 0.16-0.86% in maize kernel (Tang et al. 2013, Reddy et al. 2013, Cong et al. 2015, Bjarnason and Vasal 1992, Vivek et al. 2008). Similarly, other two essential amino acids, tryptophan and methionine range from 0.02-0.074% (Cong et al. 2015, Bjarnason and Vasal 1992, Vivek et al. 2008) and from 0.15-0.37% (Tang et al. 2013, Lair and Messing 2002), respectively. Corn oil having major role in improving the availability of fat soluble vitamins and carotenes, varies between 1.4 and 6.0%. In high oil Illinois lines oil content up to 15% is reported (Lambert et al. 1998, Enyisi et al. 2014, Tang et al. 2013, Cong et al. 2015, Ai and Jane 2016).

β-carotene, a precursor of vitamin-A having significance in terms of vision, immunity and reproduction, ranges between 3.4-21.7 mg/kg in maize kernel ((Muthusamy et al. 2014, Pillay et al. 2014). Vitamin-E plays important role as antioxidant, improving immune responsiveness and prevention of oxidation of polyunsaturated fatty acids (PUFA) which is present in maize kernel approximately in the range of 4.6-30 mg/kg (Li et al. 2012). The iron, which plays an important role as a catalyst in transporting the oxygen to red blood cells (Fe) content varies from 11.28-83.35 mg/kg in maize kernel (Agrawal et al. 2012, Prasanna et al. 2011, Mallikarjuna et al. 2014, Chakraborti et al. 2011b). Zn, an integral part of different enzymes involved in synthesis and degradation of carbohydrates, protein and lipids, range from 3.81-52.95 mg/kg in maize kernel (Chakraborti et al. 2011a, Prasanna et al. 2011, Guleria et al. 2013, Mallikarjuna et al. 2014). The anti-nutritional factor, phytic acid is a strong chelator of Fe$^{2+}$ and Zn$^{2+}$ in-vivo and lead to an insufficient bio-availability of Fe and Zn (Hunt 2003). Konietzny and Greiner (2003) reported the range of phytic acid from 0.68-14.2 mg/g in maize kernel on the dry weight basis.

**Discovery of mutants influencing maize quality traits**

In maize, the *waxy1*, the first gene influencing kernel quality was identified by Collins and Kempton in 1913. It is recessive in nature and influences kernel type with specific phenotype of dull and waxy-like appearance. Subsequently many additional genes modifying kernel appearance, including *ae1, su2, f1, fl2, du2, o2* and *bt1* were identified through genetic analysis (Hutchinson 1921, Mangelsdorf 1923, 1926). During the initial era of maize genetics, genes were identified based on the distinct morphological characters conditioned by the mutant genotype. Allelic relationship has been determined using classical genetic complementation experiment and new mutants have been assigned as novel ones or allelic to existing mutant (Coe...
1985, Jha et al. 2016). Genetic analysis using these mutants led to development of detailed genetic maps, which were further enriched with biochemical and molecular markers for identification and localization of genes governing quality traits in the linkage maps (Yang et al. 2005, Yan et al. 2010). Different endosperm mutants with enhanced quality traits as reported by the researchers have been presented in Table 2.  

Maize endosperm is constituted primarily of starch rich tissues that support embryo at germination and hence determines its nutritional quality (Balconi et al. 2007). The protein is mainly stored in maize endosperm as a group of prolamins, known as zeins. Zeins are synthesized on rough endoplasmic reticulum (ER) membranes and accumulate in the ER as insoluble accretions called protein bodies (Larkins and Hurkman 1978). Certain mutants altering zein synthesis lead to protein bodies with abnormal morphology, size, number and result in kernels with a soft and starchy texture. Mutations reducing α-zein synthesis, such as opaque2 (o2) (Mertz et al. 1964), results in small unexpanded protein bodies (Geetha et al. 1991). Mertz et al. (1964) reported that the maize endosperm's homozygous for the o2 mutant recorded different amino acid pattern than the normal maize kernel and have 69% more lysine. Than o2 mutant was identified in W22 inbred and located on chromosome 7L. Another important mutant opaque16 (o16) was identified in China. The QCL3024 (o16) and QCL3021 (o16) lysine mutant lines having opaque endosperm were derived from a self-cross population isolated from Robertson’s Mutator stock. Two F2 generations were developed, one from a cross between QCL3024 and QCL3010 (a wild type line) and another from a cross between Qi205 (o2) and QCL3021 and evaluated for lysine content. The distributions indicate

| Nutrients     | Breeding Target | References                                                                 |
|---------------|-----------------|----------------------------------------------------------------------------|
| Protein       | 9-11%           | Enyisi et al. (2014), Pedersen et al. (2014), Cong et al. (2015)            |
|               | 4.50-9.87%      |                                                                           |
|               | 6.0-12%         | Ai and Jane (2016)                                                         |
|               | 7.5-9.1%        |                                                                           |
|               | 5.0-10.8%       |                                                                           |
|               | 8.34-13.24%     | Butts-Wilmsmeyer et al. (2017)                                             |
| Lysine        | 2.5%            | Tang et al. (2013), Reddy et al. (2013), Cong et al. (2015)                 |
|               | 0.73-0.86%      |                                                                           |
|               | 0.38-0.58%      |                                                                           |
|               | 0.21-0.38%      |                                                                           |
|               | 0.16-0.26%      | Bjarnason and Vasal, (1992), Vivek et al. (2008)                           |
| Tryptophan    | 0.60%           | Cong et al. (2015), Bjarnason and Vasal (1992), Vivek et al. (2008)       |
|               | 0.036-0.074%    |                                                                           |
|               | 0.02-0.06%      |                                                                           |
| Methionine    | 0.5%            | Tang et al. (2013), Lai and Messing (2002)                                 |
|               | 0.15-0.17%      |                                                                           |
|               | 0.20-0.37%      |                                                                           |
| Oil           | 6%              | Enyisi et al. (2014), Tang et al. (2013), Cong et al. (2015)               |
|               | 2.17-4.43%      |                                                                           |
|               | 4.93-5.62%      |                                                                           |
|               | 1.4-5.0%        |                                                                           |
|               | 3.0-6.0%        | Ai and Jane (2016)                                                         |
| Starch        | 73%             | Guo et al. (2013), Cook et al. (2012), Yangcheng et al. (2013)             |
|               | 67.10-74.40%    |                                                                           |
|               | 59.60-73.00%    |                                                                           |
|               | 66.6-74.1%      |                                                                           |
| Provitamin-A  | 15 mg/kg        | Pillay et al. (2014), Muthusamy et al. (2014), Choudhary et al. (2014)     |
|               | 3.4-21.7 mg/kg  |                                                                           |
| Tocopherol    | 15 mg/kg        | McDonald et al. (1998), Li et al. (2012), Egesel et al. (2003)             |
|               | 4.6-14.8 mg/kg  |                                                                           |
| Fe            | 60 mg/kg        | Tang et al. (2013), Agrawal et al. (2012), Prasanna et al. (2011)         |
|               | 12.5-19.7 mg/kg |                                                                           |
|               | 20.38-54.29 mg/kg|                                                                           |
|               | 11.28-60.11 mg/kg|                                                                           |
|               | 16.61-83.35 mg/kg|                                                                           |
|               | 13.95-39.31 mg/kg|                                                                           |
| Zn            | 38 mg/kg        | Tang et al. (2013), Chakraborti et al. (2011a), Chakraborti et al. (2011b) |
|               | 12.5-20.9 mg/kg |                                                                           |
|               | 17.57-49.14 mg/kg|                                                                           |
|               | 21.85-40.91 mg/kg|                                                                           |
|               | 15.14-52.95 mg/kg|                                                                           |
|               | 3.81-35.83 mg/kg |                                                                           |
|               | 14.27-53.20 mg/kg|                                                                           |
| Phytic acid   | 11.5-14.2 mg/g dry weight | Konietzny and Greiner (2003)                                             |
| (anti-nutritional 1.1 mg/g factor) | 0.68-1.5 mg/g | Cong et al. (2015)                                                         |
Table 2  Details of different mutants/genes for enhancement of quality traits

| Mutants/Genes/ QTLs | Inheritance pattern | Phenotypic effect | Enzymatic activity/Mode of action | Chr. | Source | References |
|---------------------|---------------------|-------------------|----------------------------------|------|--------|------------|
| **Opaque-1 (o1)**   | Recessive           | The amount of non-zein protein in o1 was nearly identical to that in the wild type, no significant increase in lysine content. | Encodes a Myosin XI Motor Protein that affects protein body formation by disrupting ER morphology and motility. | 4L   | W64A   | Hunter et al. 2002 |
| **Opaque-2 (o2)**   | Recessive           | Different amino acid pattern, higher lysine percent, reduced zein to glutenin ratio | Encodes a defective basic-domain-leucine-zipper transcription factor, regulates several endosperm-expressed genes, particularly in 22-kDa α-zeins. | 7L   | W22    | Mertz et al. 1964; Schmidt et al. 1990 |
| **Opaque-5 (o5)**   | Recessive           | The amount of non-zein protein was nearly identical to that in the wild type, nearly 1.4% higher lysine per cent. | - | 7L | W64A5 | Hunter et al. 2002 |
| **Opaque-6 (o6)**   | Recessive           | Relatively high in lysine content, showed shift in zein to glutenin ratio, increase in non-protein nitrogen albumins and insoluble proteins | - | 8L | O6 mutant | Ma and Nelson 1975 |
| **Opaque-7 (o7)**   | Recessive           | Reduction in α-zein protein synthesis and the formation of protein bodies that are significantly smaller than normal | Encodes an acyl-activating enzyme-like protein that influences amino acid and zein protein synthesis | 10L  | W22    | Wang et al. 2011 |
| **Opaque-9 (o9)**   | Recessive           | Nearly 1.4% higher lysine content | - | 5L | W64A9 | Hunter et al. 2002 |
| **Opaque-10 (o10)** | Recessive           | Controls Protein Bodies morphology in maize endosperm | Encodes a novel cereal-specific Protein Bodies protein, is essential for the ring-shaped distribution of 22-kD and 16-kD zeins | 7L | MGN-25:969-5 | Dannenhoffer et al. 1995 |
| **Opaque-11 (o11)** | Recessive           | Increased non-zein protein, 1.8 times higher lysine | - | W64A | Hunter et al. 2002 |
| **Opaque-12 (o12)** | Recessive           | Thin, varied size, scarred mutant kernels that produce chlorophyll-deficient plants | - | 4S | ox-7638 | Nelson 1981 |
| **Opaque-13 (o13)** | Recessive           | The mutant kernels are etched and may have a thin rim of coarse starch on the abgerminal side | - | 1S | ox-7729 | Nelson 1981 |
| **Opaque-15 (o15)** | Recessive           | 2- to 3-fold reduction in γ-zein mRNA and protein synthesis | Alters the ratio of mRNAs encoded by the A and B γ-zein genes | 7L | MGN-25:969-5 | Dannenhoffer et al. 1995 |
| **Opaque-16 (o16)** | Recessive           | o16 along with o2 increases lysine by 30% over o2o2 or o16o16 alone | - | 8L | Robertson’s Mutator stocks | Yang et al. 2005 |
| Mutator-tagged opaque-140 (mto140) | Recessive | mto140/arohdi-1 seeds shows a general reduction in zein storage protein accumulation and an elevated lysine due to disruption in amino acid biosynthesis. | - | W64A (BC6) | Holding et al. 2010 |
| **Floury-1 (fl-1)** | Semi-dominant       | Mutant fl-1 do not manifest significant increase in Lysine as o2 but increase the methionine content. | Encodes an ER membrane protein involved in facilitating the localization of 22-kD α-zein in the protein bodies | 2L  | W64Af1-Mu1 | Hays and East 1915 |

Contd.
| Mutants/Genes/ QTLs          | Inheritance pattern | Phenotypic effect                                                                                                                                                                                                                                                                                                                                 | Enzymatic activity/Mode of action                                                                                                                                                                                                 | Chr | Source          | References          |
|-----------------------------|---------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|------------------|--------------------|
| *Floury-2* (*fl-2*)         | Semi-dominant       | Formation of a soft, starchy endosperm with a reduced amount of prolamin (zein) proteins and twice the lysine content of the wild type.                                                                                                                                                                                                              | Associated with small, irregularly shaped protein bodies, elevated levels of a 70-kDa chaperone in ER and a novel 24-kDa polypeptide in zein fraction.                                                                                     | 4S  | W64Afl2         | Nelson *et al.* 1965; Coleman *et al.* 1997 |
| *Floury-3* (*fl3*)          | Semi-dominant       | Relatively high in lysine content, showed shift in zein to glutenin ratio.                                                                                                                                                                                                                                                                                                                                   | -                                                                                                                                                                                                                                           | 8L  | fl-3            | Ma and Nelson 1975 |
| *Floury-4* (*fl4*)          | Semi-dominant       | Reduction in α-zein protein synthesis and the formation of protein bodies that are significantly smaller than normal.                                                                                                                                                                                                                                      | Defective signal peptide in a z1A 19-kD α-zein                                                                                                                                                                                               | 4S  | 5512G           | Wang *et al.* 2014 |
| *Mucuronate* (*Mc*)        | Dominant            | 1. 4 times higher lysine.                                                                                                                                                                                                                                                                                                                                                                                      | Mc encodes a 16-kD γ -zein with a frame shift mutation                                                                                                                                                                                            | 2L  | B37Mc           | Salamini 1981; Hunter *et al.* 2002 |
| Defective endosperm B30     | Dominant            | Increased non-zein protein, 1. 8 times higher lysine                                                                                                                                                                                                                                                                                                                                                           | De-B30 is a 19-kD α-zein with a single amino acid replacement resulting in a defective signal peptide                                                                                                                                   | 7S  | B37De *-30       | Salamini 1981; Hunter *et al.* 2002 |
| *Zps10*/*Zpr10*             | -                   | Zps10/(22) is structural gene and Zpr10/(22) is regulatory gene                                                                                                                                                                                                                                                                                                                                               | Overproduction of the zein protein by trans-acting mechanism                                                                                                                                                                                      | 9 & | BSSS53, W23 and B73 | Benner *et al.* 1989 |
| *dzrl*                      | -                   | 30% phenotypic variability explained                                                                                                                                                                                                                                                                                                                                                                        | Overproduction of a methionine-rich, 10-K zein                                                                                                                                                                                                  | 4   | (MO17 × BSSS53) × MO17 | Chaudhuri and Messing 1995 |
| *amylose extender1* (*ael*)| Recessive           | 66% high amylose than normal endosperm                                                                                                                                                                                                                                                                                                                                                                       | Branching enzyme II                                                                                                                                                                                                                              | 5   | A636 × B73 | Vineyard and Bear 1952 |
| *brittle1* (*bt1*)          | Recessive           | Shrunken endosperm of brittle texture, slightly darker than normal                                                                                                                                                                                                                                                                                                                                          | Adenylate translocator                                                                                                                                                                                                                             | 5   | Improved         | Mangesdorf1926 |
| *brittle-2* (*bt2*)         | Recessive           | Shrunken endosperm of brittle texture, slightly darker than normal                                                                                                                                                                                                                                                                                                                                        | ADP-Glc pyrophosphorylase                                                                                                                                                                                                                         | 4   | -               | Teas and Teas 1953 |
| *dull1* (*dul*)             | Recessive           | 42% high amylose than normal endosperm                                                                                                                                                                                                                                                                                                                                                                      | Starch synthase II                                                                                                                                                                                                                                 | 10  | Surcropper       | Mangesdorf1947 |
| *shrunken1* (*sh1*)         | Recessive           | Highly collapsed, opaque and brittle and have a weight of 75% that of normal endosperms                                                                                                                                                                                                                                                                                                                       | Sucrose synthase                                                                                                                                                                                                                                   | 9   | Kansas           | Hutchison1921 |
| *shrunken-2* (*sh2*)        | Recessive           | Shrunken, opaque to translucent                                                                                                                                                                                                                                                                                                                                                                           | ADP-Glc pyrophosphorylase                                                                                                                                                                                                                         | 3   | Natural mutant  | Mains 1949 |

**Table 2. (Continued)**

Characterization of Mutants/Genes/
QTLs & Properties

**Starch**

| Mutants/Genes/ QTLs          | Inheritance pattern | Phenotypic effect                                                                                                                                                                                                                                                                                                                                 | Enzymatic activity/Mode of action                                                                                                                                                                                                 | Chr | Source          | References          |
|-----------------------------|---------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|------------------|--------------------|
| *amylose extender1* (*ael*)| Recessive           | 66% high amylose than normal endosperm                                                                                                                                                                                                                                                                                                                                                                       | Branching enzyme II                                                                                                                                                                                                                              | 5   | A636 × B73 | Vineyard and Bear 1952 |
| *brittle1* (*bt1*)          | Recessive           | Shrunken endosperm of brittle texture, slightly darker than normal                                                                                                                                                                                                                                                                                                                                          | Adenylate translocator                                                                                                                                                                                                                             | 5   | Improved         | Mangesdorf1926 |
| *brittle-2* (*bt2*)         | Recessive           | Shrunken endosperm of brittle texture, slightly darker than normal                                                                                                                                                                                                                                                                                                                                        | ADP-Glc pyrophosphorylase                                                                                                                                                                                                                         | 4   | -               | Teas and Teas 1953 |
| *dull1* (*dul*)             | Recessive           | 42% high amylose than normal endosperm                                                                                                                                                                                                                                                                                                                                                                      | Starch synthase II                                                                                                                                                                                                                                 | 10  | Surcropper       | Mangesdorf1947 |
| *shrunken1* (*sh1*)         | Recessive           | Highly collapsed, opaque and brittle and have a weight of 75% that of normal endosperms                                                                                                                                                                                                                                                                                                                       | Sucrose synthase                                                                                                                                                                                                                                   | 9   | Kansas           | Hutchison1921 |
| *shrunken-2* (*sh2*)        | Recessive           | Shrunken, opaque to translucent                                                                                                                                                                                                                                                                                                                                                                           | ADP-Glc pyrophosphorylase                                                                                                                                                                                                                         | 3   | Natural mutant  | Mains 1949 |

Contd.
| Mutants/Genes/ QTLs | Inheritance pattern | Phenotypic effect | Enzymatic activity/Mode of action | Chr. | Source | References |
|---------------------|---------------------|-------------------|----------------------------------|------|--------|------------|
| shrunken-4 (sh4)    | Recessive           | Shrunken and opaque| -                                | 5    | Sh4/ Sh4| Tsai & Nelson 1969 |
| sugary1 (su1)       | Recessive           | Increased amounts of amylose and intermediate fractions compared with normal starch. | Isoamylase | 4    | Natural mutant | Correns 1901 |
| sugary-2 (su2)      | Recessive           | Slightly tarnished to tarnished                              | -                                 | 6    | -      | Eyster 1934 |
| waxy-1 (wx1)        | Recessive           | Increases sugars and water-soluble polysaccharides           | Starch granule bound starch synthase| 9    | Sanford white | Collins 1909 |

**Provitamin-A**

| Y1 (Psy1)           | Dominant            | conversion of white maize to yellow/orange                  |                                 | 6L   | Q60    | Buckner et al. 1990 |
| crtRB1              | Recessive           | 7-27% phenotypic variability explained                       | Hydroxylation of α- and β-carotene into non-provitamin A carotenoids | 10   | B73 × BY804 | Yan et al. 2010 |
| lcyE                | Recessive           | -                                                              | Converts more lycopene to the β, ε branch and produce α-carotene and lutein | 8    | Q×47   | Harjes et al. 2008 |

**Vitamin-E**

| VTE4                | Recessive           | Increase 3.2 fold vit. E compared to normal maize            | Encodes γ-tocopherol methyltransferase which is involved in the rate limiting conversion of γT to αT | 6L   | Q60    | Li et al. 2012 |

**Low phytic acid**

| lpa1-1              | Recessive           | 66% reduction in seed phytic acid phosphorus                 | Reduced phytate matched solely by increased inorganic P. Viable | 1S   | M2 progeny of 90046-13 | Raboy et al. 2001 |
| lpa2-1              | Recessive           | 50% reduction in seed phytic acid phosphorus                 | Reduced phytate matched by increased inorganic P and increases in other Inositol phosphates. Viable but plant and seed effects. | 1S   | M2 progeny of 90041-1, 90041-4, and 90041-12 | Raboy et al. 2001 |
| lpa241              | Recessive           | 90% reduction of phytic acid and about a tenfold increase in seed-free phosphate content | "Ins-supply" pathway and the later Ins phosphate/Ptd Ins-phosphate pathway that converts INS to INS P6 | 1S   | ACR seed stock (mutant) | Pilu et al. 2003 |
| lpa3                | Recessive           | 50% less phytic acid than the corresponding wild-type        | Role in myo-inositol and MIK in phytic acid biosynthesis in developing seeds | 1S   | M2 progeny lpa-061326 | Shi et al. 2005 |
that the lysine content in the two populations is regulated by the major gene of o16 and genes of o2 and o16 (double mutant), respectively. The o16 alone possesses lysine and tryptophan that are as high as o2 and it does have influence on opaqueness (Sarika et al. 2016). The pyramiding of o16 mutant with o2 led to higher accumulation of lysine and tryptophan (Yang et al. 2005, Sarika et al. 2018). A new mutant, opaque15 (o15), reported 2-3 fold reduction in γ-zein mRNA. On phenotype basis, o15 appears to be a mutant of an o2 modifier gene (Dannenhoffer et al. 1995). The mutant floury-2 (fl2) identified by Nelson et al. (1965) had lysine content equal to mutant o2. But it also had enhanced methionine concentration than in any other stock tested. Likewise several mutants have been identified that can alter the zein synthesis and increase the protein in maize. The major reason for these changes is the synthesis of proteins with a greater content of basic amino acids in the acid-soluble fraction of the mutant endosperm. This is accompanied by a reduction in the ratio of zein to glutenin.

In contrast to above mentioned recessive mutations, a dominant mutation DeB-30 influencing protein quality is also reported in maize (Salamini et al. 1979). It contains 50% more lysine than the normal maize but linked with reduction of seed weight limiting its practical utilization in breeding for quality improvement. Another dominant mutant Mc (Salamini et al. 1983)interferes with the synthesis of storage proteins in the endosperm and results in enhanced level of methionine. Several endosperm mutants (at least 18 such mutants) effecting kernel phenotypes (brittle texture) and grain quality (susceptibility to insect pests, and inferior functional characteristics of products made from their flour) by altering maize starchy have been identified, but only for very few mutants, molecular basis of the mutation is well characterized (Hunter et al. 2002).

Several mutants affecting starch synthesis pathway can alter the level of amylose and amylopectin as well as sugar content in the kernel. The mutants, viz. sugary 1 (su1), shrunken 1 (sh1), amylose extender 1 (ae1), brittle 1 (bt1) and waxy 1 (wx1) were discovered by Correns (1901), Hutchison (1921), Vineyard and Bear (1952), Mangelsdorf (1926) and Collins (1909), respectively. Among these, su1 and sh1 have been extensively used worldwide for development of sweet corn cultivars. Mutant su1 is a mutated version of gene influencing starch debranching enzyme. This enzyme is responsible for enhancing the water soluble polysaccharides, reducing sugars and sucrose in milky to ripening stage and decrease starch accumulation in mature kernels, resulting in sweet kernels (East and Hayes 1911, Dinges et al. 2001). Recessive mutant, se 1 is a modifier of su 1 and enhances sugar level in maize kernels (Ferguson et al. 1978). Another gene for sweetcorn, sh2 as identified by Mains (1949), encodes the large subunit of the starch biosynthetic gene, adenosine diphosphate glucose pyrophosphorylase (AGPase). This enzyme plays a crucial role in starch biosynthesis (Hannah and Nelson 1976, Bhave et al. 1990, Lee et al. 2009, Hannah et al. 2012). Among the genes influencing sugar content su1 and sh2 have been used quite extensive in sweet corn breeding, however relatively few studies have been conducted on combining sul and sh2 in a single genetic background. A study conducted at ICAR-Indian Agricultural Research Institute (IARI), New Delhi led to the development of array of diverse sweet corn inbreds in the genetic background of sul1su1, sh2sh2 and su1su1/sh2sh2 but it resulted in generation of few promising sweet corn hybrids (Hossain et al. 2013).

Pro-vitamin A is another important nutrient element of human diet for which considerable natural variation is present in maize germplasm. Based on combined approach of association analysis, linkage mapping, expression analysis and mutagenesis, it has been found that the favourable alleles of ceyE locus alter flux down carotene versus β-carotene branches of the carotenoid pathway and can enhance pro-vitamin A content up to three-fold in maize endosperm (Harjesh et al. 2008). Another major QTL for pro-vitamin A, viz. criRB1 has been mapped which significantly enhances beta-carotene content by blocking its conversion to abscisic acid (ABA) (Yan et al. 2010).

The genes governing the level of anti-nutritional factor phytic acid have also been identified and lpa1-1 was the first mutant allele identified in M2 segregating generation of 90046-13, which reduces the phytic acid by 50 to 60% in seed but total phosphorous is unaltered (Raboy et al. 2001). The decrease in phytic acid in mature lpa1-1 seeds is resultant of corresponding increase in inorganic phosphate (Pi). In the mature lpa2-1 seed it is accompanied by increases in Pi and at least three other myo-inositol (Ins) phosphates. In both cases the sum of seed Pi and Ins phosphates is constant and similar to that observed in normal seeds. Homozygosity for either mutant results in a seed dry weight loss, ranging from 4 to 23% (Pilu et al. 2003).

There are no specific single genes discovered for high oil, methionine and micronutrients (Fe & Zn) but several minor effect QTLs have been reported by different researchers (Table 3).

**Classical to molecular approaches for quality breeding in maize:** In classical plant breeding relying on phenotypic selection for quality trait remained effective historically. However, for quality traits, indirect selections based on morphological traits have remained largely ineffective because of lack of definite correlation between quality traits and morphological characteristics. Most of the quality traits in maize are governed by recessive genes (Mertz, et al. 1964). Hence, stringent control of pollination is required while handling quality related breeding material and the selection can be made on the basis of biochemical evaluation rather than phenotypic selection. Further, recessive genes can be selected for only in homozygous state because every backcrossing calls for one cycle of selecting to select the desirable segregants. Hence, backcross breeding turns out to be time taking. Further, presence of modifiers as in the case of o2, the task become further complicated. A combination of more than one nutritional trait i. e. pyramiding nutritional traits is a
Table 3. Detail of QTLs influencing different quality traits in maize

| QTL/Gene | Mapping population (size) | Parentage | Linked markers | Marker type | Chromosome location | PVE (%) | Ref |
|----------|---------------------------|-----------|----------------|-------------|---------------------|---------|-----|
| Protein, oil and starch concentration/composition |
| 74 loci (SNPs) for oil biosynthesis (including 26 for oil concentration) | Diverse Panel 508 (AM508, 473 regular and 35 high-oil lines) | SNP/G/T Indel_8 Indel_146/472 Indel_2000 Indel_20 | SNPs (Illumina Maize SNP50 Bead Chip) | 1, 2, 4, 6, 8 and 9 | 83 (26 loci for high oil) | Li et al. 2012 |
| 15 QTLs (palmitic, stearic, oleic, linoleic, and linolenic acids) | BC$_3$S$_1$ (150) qOHL06-1 and qPRO06-1 | IHO \( \times \) B73 | umc1006 | SSRs | 6 | 10.9-39.6 | Wassom et al. 2008a |
| Several QTLs for kernel oil, protein, and starch | BC$_3$S$_1$ (150) | IHO \( \times \) B73 | - | SSRs | 6 | 36.7 (Oil) | Wassom et al. 2008b |
| 11 QTLs (kernel oil concentration) and 10 QTLS (kernel protein concentration) | Zheng 58 (low oil and protein) \( \times \) B73 (high oil and protein) | umc1904- phi100175 umc1272- bnlg1839 umc1019-umc2038 umc219-umc2243 | SSRs | 1, 2, 3, 4, 5, 6, 7, 8 and 10 | 4.6 to 11.1 (Oil) | Yang et al. 2016 |
| 17 QTLs (6, 6 and 5 for kernel oil, protein and starch concentrations, respectively) | By804 \( \times \) B73 | bnlg2086-umc1122 phi96100-umc1422 umc1562-umc1960 | SSRs | 1, 2, 4, 5, 6, 7, 8 and 9 | 4.3-13.1 (Oil) 5.19-6.66 (Protein) 4.1-7.9 (Starch) | Zhang et al. 2008 |
| 12 and 6 QTLs (4 and 2 QTLs for starch, 4 and 3 QTLs for protein and 4 and 1 QTL for oil) in F$_{2,3}$ and BC$_3$F$_2$ populations, respectively | Dan232 (dent com) \( \times \) N04 (popcorn) | umc1269-umc1948 umc1112-umc1936 umc1403-phi001 | SSRs | 1, 3, 4, 5, 6, 7, 8 and 9 | 5.2-10.6 (Starch) 5.14-3.4 (Protein) 6.2-8.5 (Oil) | Yang et al. 2008 |
| 16 single-population and 19 joint-population QTLs for protein and 21 QTLS for protein-oil | KY220 \( \times \) 8984 KY220 \( \times \) 8622 | umc2075-bnlg2046 bnlg1325-bnlg1523 | SSRs | 3, 5, 6, 7, 8 and 9 | 4.4-13.4 | Yang et al. 2014 |
| 12 and 14 QTLs for oil in two populations, respectively | KY220 \( \times \) 8984 KY220 \( \times \) 8622 | umc2316-umc1979 | SSRs | 1, 3, 4, 5, 6, 8 and 10 | 3.93-14.47 | Yang et al. 2012 |
| 58 QTLs (kernel oil content, embryo quality related traits) | By804 \( \times \) B73 | Q8-umc1979 | SSRs, Indels & Candidate gene markers | 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 | 1.1-20.5 | Yang et al. 2012 |

Contd.
### Table 3 (Concluded)

| QTL/Gene | Mapping population (size) | Parentage | Linked markers | Marker type | Chromosome location | PVE (%) | Ref       |
|----------|---------------------------|-----------|----------------|-------------|---------------------|---------|-----------|
| 3 Major QTLs for carotenoids (β-carotene, β-cryptoxanthin) | F2:3 (200) Testcross families (185) | W64a × A632 F2:3 families × AE335 y1SSR–bnlg249 zdsRFLP-phi034 bmc1176-bmc1599 | SSR, STS and CAPS | 6, 7 and 8 | 11.8-25.4 | Wong et al. 2003 |
| 31 QTLs for carotenoids | RILs (203) | By804 × B73 Y1ssr–ume1595 ymca2313–Y1ssr | SSR, STS and CAPS | 1, 3, 5, 6, 7, 8 & 10 | 6.6-27.2 | Chander et al. 2008 |
| Several QTLs for γT, αT, TT and α/γ | F2:4 | W64a × A632 F2:4 families × AE335 phi085 | SSR | 1 and 5 | - | Wong et al. 2003 |
| 31 putative QTL for tocopherol content and composition | RILs (208) | By804 × B73 umc1075–ume1304 umc1598–bnlg1811 bnlg1792–phi091 | SSR, STS, CAPS, and AC-PCR | 1, 2, 3, 5, 6, 7, 8, 9 and 10 | 29.32 (γ-T) 15.54 (α-T) 18.40 (δ-T) | Chander et al. 2008 |
| 30 QTLs with 3 major QTLs (qdl-1, qc5-1/qd5-1 and qc5-2) | F2:4 (237) F2:4 (218) | K22 × CI7 K22 × Dan340 PZA02117.1-PHM4926.16 PZA00352.23-PZA02060.1 | SNPs (GoldenGate assay) | 1 and 5 | 53 (αT) 30 (αT) 25 (αT) | Shutu et al. 2012 |
| Major QTL ZmVTE4 (α-tocopherol variation) | Association panel (543 inbred lines) | Diverse panel | InDel7, InDel118, SNP25815 bnlg1237 & phi085 | SNPs (Illumina) | 5 | 33 | Li et al. 2012 |
| Major QTL ZmVTE2 | F2:3 | N6xNC296 | SNPs | 9 | 22 | Fenton et al. 2018 |
| 27 QTLs | RILs | B73×Mo17 | mmp 144, rz87 ay110452, ay110625 and mmp 125 | SSRs | 9 & 10 | 4-46 | Baxter et al. 2013 |
| 3 and 10 QTLs for grain Fe RILs concentration and bioavailability, respectively | B73×Mo17 | mmp 144, rz87 and sh1 (Fe concentration) psr754b, php20528 and umc 2134 (Fe Bioavailability) | SSRs | 3, 6 and 9 | 26 and 54 respectively | Lunga’ho et al. 2011 |
| 3 co-localized QTLs for Fe, Zn, Mg and P | B84×OS6-2 | bnlg1456 | SSRs | 3 | 24.10 and 22.40 for Fe and Zn respectively | Simic et al. 2011 |
more desirable strategy for quality enhancement than to improve individual trait in separate genetic backgrounds. Deployment of phenotypic selection for multiple quality trait improvement simultaneously, possesses financial and operational challenges as it is in terms of cost, time and labour. Furthermore, plant breeders need to combine a suite of traits in a single cultivar, which may limit gains from phenotypic selection.

These constraints of conventional breeding can be overcome through molecular breeding that helps to study genetic diversity, characterize genetic architecture of germplasm and thereby enhancing the efficacy of selection (Moose and Munm 2008). Molecular markers have also been successfully harnessed for mapping of QTLs for quality traits in maize. Various studies on identification or mapping of major genes/QTLs and minor QTLs have been carried out in recent past (Table 4). Desirable mutants having major effects have proved vital role in the nutritional improvement programmes.

The protein quality attributing genes, viz., o2 and a16 genes for high lysine content were mapped through molecular markers by Babu et al. (2005) and Yang et al. (2005) respectively on chromosome 7S and 8L. Markers Assisted Selection (MAS) can help to combine the genes for quality traits through marker assisted gene pyramiding approach thereby providing more acceptable alternative of normal maize in the form of multi-nutri-maize (multiple nutrient rich maize). Zhang et al. (2013) introgressed two genes, viz., o2 and o16 for increasing lysine content in waxy line QCL5019 from 0.28% to more than double 0.62% in introgressed families. The maize hybrid, Vivek QPM 9 released in 2008 having enhanced lysine and tryptophan as per its non-QPM version of Vivek 9 is the first successful example of commercial release of MAS-derived maize hybrid in India (Gupta et al. 2013). Similarly, further effort for utilizing MAS for enhancing provitamin A levels in Vivek QPM 9 resulted into provitamin A maize hybrid Vivek QPM9 Improved (Muthusamy et al. 2014). It possesses both o2 and crtRB1 genes and therefore multinutrient rich. Zunjare et al. (2018) further successfully combined both crtRB1 and lcyE in the genetic background of four popular QPM hybrids, HQPM-1, HQPM-4, HQPM-5 and HQPM-7. The products (inbreds/hybrids) with enhanced quality and developed through MAS has been detailed in the Table 4.

There are different genotypes or cultivars developed based on sweet corn mutants, viz., Boston, Bonus and Jubilee (su1-based), Anava, Champ and Dallas (se-based) and Candle, challenger and Sheba (sh2-based). Other than these the genotypes developed based on the different combinations of sh2, su1 and se genes have been developed, viz., IL27a, I453 and P39 (su1su1/Sh2Sh2), IL677a (su1su1/se1se1/Sh2Sh2) and EPS18 (Su1Su1/se1se1/sh2sh2

| Trait(s) improved | Gene(s) introgressed | Marker name | Marker type | Inbred/hybrid | Country | Reference |
|------------------|---------------------|-------------|-------------|---------------|---------|-----------|
| QPM              | opaque2             | phi057, phi112 and umc1066 | SSR | Inbred | India | Babu et al. 2005 |
| QPM              | opaque2             | phi057, phi112 and umc1066 | SSR | Inbred | Uganda | Manna 2005 |
| QPM              | opaque2             | phi057, phi112 and umc1066 | SSR | Inbred | Kenya | Danson et al. 2006 |
| QPM              | opaque2             | phi057     | SSR | Inbred & hybrid | Thailand | Jompuk et al. 2011 |
| QPM              | opaque2             | phi057, phi112 and umc1066 | SSR | Inbred | India | Gupta et al. 2013 |
| QPM              | Opaque2             | umc1066 and phi057 | SSR | Hybrid | India | Hossain et al. 2018 |
| QPM              | opaque2             | phi057 and umc1066 | SSR | Inbred | Serbia | Kostadinovic et al. 2014 |
| QPM              | opaque2             | Phi057     | SSR | Inbred | Philippines | Magulama and Sales 2009 |
| QPM              | opaque16            | umc1141 and umc1121 | SSR | Inbred | India | Yang et al. 2013, Zhang et al. 2010 |
| QPM              | opaque16            | umc1141 and umc1149 | SSR | Inbred | India | Sarika et al. 2016 |
| QPM              | opaque2 & opaque16  | (phi057, phi112 and umc1066) (phi027 and phi112) | SSR | Inbred | India | Zhang et al. 2013, Sarika et al. 2018 |
| ProA             | crtRB1              | umc1066, crtRB1-3′TE-F, crtRB1-3′TE-R1 and crtRB1-3′TE-R2 | SSR | Inbred & hybrids | India | Muthusamy et al. 2014 |
| ProA             | crtRB1              | crtRB1-5′TE-2 and crtRB1-3′TE-1 | SSR | Inbred | China | Liu et al. 2015 |
| ProA             | crtRB1 & lcyE       | Phi057 and InDel 3′TE and 5′TE | SSR | Inbred & hybrid | India | Zunjare et al. 2018 |
| Vitamin-E        | VTE4                | InDel7 and InDel118 | Functional markers | Inbred | China | Feng et al. 2015 |
| Low phytate      | lpa2                | umc2230     | SSR | Inbred | India | Tamilkumar et al. 2014, Sureshkumar et al. 2014 |
based) have been developed (Revilla et al. 2006, Szymanek et al. 2015).

The high oil populations IHO, SHO, DHO, ALHO, ASK, ALEX synthetic, KYHO and hybrids, viz. Illinois 6021, 6052, 6001 and Burr white have been developed (Hopkins 1899, Wang et al. 2009). These have been developed through cyclic selection of high oil lines.

Amylose is a linear macromolecule which contains glucose units with α-1, 4 linkages in which each macromolecule contained one reducing end and one non-reducing end. Amylose of high-amylose corn starch has a high degree of polymerization (Takeda et al. 1989). High amylose containing maize commonly known as amylo-maize possesses more than 50% amylose contents. Amylo-maize lines, viz. H99ae, OH43ae, B89ae, B84ae and GEMS-0067 lines have been reported (Li et al. 2008). The branched component of starch is amylopectin. The iodine uptake by the branched amylopectin in high amylopectin lines (waxy lines) increase at low temperature (Banks and Greenwood 1975). For example, the iodine binding capacity of waxy maize amylopectin is 0.17 at 20°C, and 0.15% at 1.5°C. Waxy corn is a popular choice in the entire South-East Asia (Devi et al. 2017). Several landrace accessions with high amylopectin are available and used as part of food (Park et al. 2008, Liet and Thinh 2009, Bao et al. 2012, Zheng et al. 2013). Besides several hybrids with high amylopectin have been developed (Zhang et al. 2013, Yang et al. 2013).

Challenges and future perspectives: Biofortification of maize with quality traits is an essential feature to address nutritional severity. Genomics and marker assisted selection (MAS) technology has opened new avenues for improvement of complex quality traits. Following this rate a wide array of biofortified maize with high lysine, tryptophan and provitamin A has been developed. However, biofortification process and biofortified maize are associated with number of issues; some of the issues are discussed here.

First of all, enhanced protein and its content is negatively associated with grain yield (Bjarnason and Vasal 1992). Modifications of other quality traits are as such not associated with yield loss. The biggest challenge in developing cultivars with improved quality traits lies with modification of the quality through alteration of genes involved in multiple

Fig 1 MAS strategy for utilization of recessive genes for development of multi-nutrient maize.
metabolic pathways without compromise with grain yield (Collard and Mackill 2008). Hence, this is not applicable in case of sweet corn as the grain as such is not the end use. Earlier there was several QPM based hybrids released in India, viz., Shaktiman series and Protina but major drawback of these hybrids yield was less as compared to normal hybrids. QPM version of Vivek hybrid 9, Vivek QPM-9 possesses similar grain yield potential as that of the original hybrid (Gupta et al. 2013). Hosain et al. (2018) also reported similar grain yield potential of Pusa HM4 Improved, Pusa HM8 Improved and Pusa HM9 Improved with their original non-QPM version, viz., HM4, HM8 and HM9. In other study, MAS-derived versions of HM-4, HM-8, Vivek QPM-9 and Vivek Hybrid-27 for β-carotene evaluated by Muthusamy et al. (2014) at two different locations of India found that β-carotene-rich version of original hybrids were similar for grain yield potential. Hence, it may be concluded that, quality enhanced as maize cultivars can be developed without any yield penalty. Thus, plant breeders need to add nutrition as an objective to their breeding programs.

Second challenge comes in terms of commercialization of biofortified products. There are two aspects in this regards convincing the farmers. Firstly, the farmers need to be convinced of the benefits of growing and consumption of the products, and secondly the economic benefits associated with growing such products. To convince the farmers a strong extension service is needed. This can be addressed by launching ground level awareness campaign about health benefits related to its consumption. The perception of people about low yield potential of quality fortified maize is to be changed. Food processing industries needs to be linked with quality maize cultivation to harness its good benefit. Policy intervention is needed to encourage quality maize cultivation at appreciable support price. Nutrition education campaigns that effectively empower caregivers with knowledge about the importance of nutrients in health would help in a great way. Provitamin A-biofortified sweet potato projects in Kenya and Mozambique have documented the effectiveness of appropriate nutrition education. They employed community theatre, group demonstration sessions, and radio programs, in creating demand for such fortified products (De Groote et al. 2010). In Zambia, the HarvestPlus project is working closely with the Ministry of Health, the National Food and Nutrition Commission, the Ministry of Agriculture and Cooperatives (extension service), and others to develop nutrition education strategies to create lasting demand for provitamin A biofortified maize and other sources of vitamin A (De Groote et al. 2010). Such an integrated awareness campaign is certainly needed to harness the benefits of biofortified maize.

Public awareness campaigns exploiting the power of the media and national public health experts to highlight micronutrient deficiencies and promote adoption of nutritious crops, would help in the dissemination of the technology. It will also be important to communicate not only with allies or those who are undecided, but also with opponents of biofortification to ensure that they are well informed before influencing their constituents. Groote et al. (2010) reported that the adoption of QPM cultivars by the farmers varied a lot among East African countries with 70% adoption in Uganda and 30% adoption in Tanzania, while Kenya reported none. Besides the knowledge of nutritional benefit of QPM, the response of farmers’ participation in extension activities and reliable supply of good quality seeds were the important factors for the successful adoption. A study in Zimbabwe by Stevens et al. (2008) revealed that ~94% of the respondent agreed to consume yellow maize instead of traditional white maize, if educated on health benefits.

The third challenge is since the most of gene(s) conferring quality enhancement are of recessive in nature, maintenance of quality attributes under farmers’ field is difficult due to out-crossing with non-fortified maize pollen from neighboring fields. Therefore, it is needed to implement ‘biofortified maize village concept’ on the pattern of seed village concept to ensure and optimize nutritive advantages associated with recessive gene-based biofortified maize (Groote et al. 2010; Gupta et al. 2015). The biofortified maize technology propagated through a village concept can only pass on the benefit of the technology consumers and farmers. This could be accepted by the next generation of farmers through strong policy interventions. Such interventions will also strengthen community-based seed production. Seed village concept would help to produce quality seeds by mitigating the outcrossing and enrich availability of quality seed at local level.

Fourthly, the effects of different micronutrients, viz., lysine, tryptophan, provitamin A, Fe and Zn are phenotypically invisible on grains. In this case it is difficult to convince the traders about the quality standards of the farm produce of fortified maize grains. Considering the lack of availability of rapid detection kits, there is need to develop such portable rapid detection kits to detect the quality of produce and thereby assuring good price to the farmers based on the extent of the quality. A new method using a ‘proprietary formulation’ developed at Indian Institute of Maize Research, Ludhiana completes hydrolysis of maize endosperm proteins in 30 minutes (unpublished results/personal communication). The new methodology needs to be converted into a kit which is expected to drastically shorten the time required for tryptophan estimation, and can be used for estimating other amino acids as well. Conscious observation of food habit of people, industry is coming up with newer food products frequently. Increased awareness of people through internet is pushing industry to provide novel options to cater their needs. Corn flakes, as a nutritionally rich breakfast is a classical example. Collaboration with industries is thus vital to develop such products from biofortified maize grains that can benefit both industry and consumer. Maize is the main component of maize-soybean feed mixture which is the major poultry feed across the globe. A deficit of essential amino acids in such feed mix has long been supplemented through addition of synthetic amino acids which raises the cost of feed making
the profits sensitive to price fluctuation of final produce. Synthetic supplements are continuously being used despite the availability of maize cultivars with balanced quality of protein. A systematic approach need to be adopted to sensitize entrepreneurs engaged in feed sector to adopt cost saving biofortified cultivars.

Finally the focus on the enhancement of yield in normal maize with less emphasis on quality from the beginning of maize breeding has resulted into the narrow genetic base germplasm for quality improvement. In the past although the recurrent selection programmes resulted into development of high oil and high protein population but progress was at very slow rate. The problem of malnutrition has sensitized plant breeders to focus on biofortified varieties. The molecular marker technology has also boosted the quality improvement programme through rapid introgression of favorable gene(s) into high yielding commercial cultivars. The maize breeding programmes needs to be strengthened through developing heterotic pools for quality traits and thereby developing high yielding nutritious maize hybrids. The new breeding technologies, viz. marker assisted recurrent selection (MARS) and genomic selection (GS) open the door to assist for enhancement and confirming the quality nutrients in maize with short period in current era.

The worldwide significant impact of biofortification, recognized by public officials is key to the success of biofortified crops. The declaration of remunerative price through minimum support price and/or premium price for biofortified maize grains in the market will encourage the farmers to grow more biofortified maize. Easy loan and subsidy to village level entrepreneurs to initiate small-scale enterprises for the development of biofortified maize-based processed food products would help in their greater dissemination. An integrated approach involving promising cultivars, extension agencies, products value addition, policy support would be important key to success of biofortified maize cultivation.

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