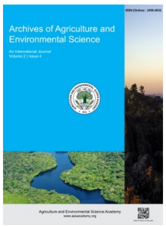
Archives of Agriculture and Environmental Science 6(1): 100-107 (2021)
https://doi.org/10.26832/24566632.2021.0601013

e-ISSN: 2456-6632

This content is available online at AESA

Archives of Agriculture and Environmental Science

Journal homepage: journals.aesacademy.org/index.php/aaes



REVIEW ARTICLE

Bio-fortified maize: Cornerstone in plant breeding to combat hidden hunger in developing countries

Damodar Gautam[1*], **Bandana Shrestha**[2] **and Bhuwan Subedi**[3]

[1]National Maize Research Program (NMRP), Chitwan, Nepal [2]GP Koirala College of Agriculture and Research Center, NEPAL
[3]Agriculture and Forestry University (AFU), Chitwan, NEPAL
[*]Corresponding author's E-mail: gautam.damodar.np@gmail.com

ARTICLE HISTORY

Received: 20 December 2020
Revised received: 07 March 2021
Accepted: 20 March 2021

Keywords

Bio-fortification
Malnutrition
Provitamin A and Zn

ABSTRACT

Malnutrition has been one of the major global health problems mainly in underdeveloped and developing world causing massive economic damage as well as distressing human life. Deficiency of useful micronutrients like vitamins and minerals including low level of availability of better quality protein causes hidden hunger which can be alleviate with the help of genetic bio-fortification of crops. Besides all the challenges, biofortified maize crops like quality protein maize along with the provitamin A and Zn hold a great future to address the malnutrition challenge combating the deficiency of malnutrtients. This is the most sustainable, cost-effective and potentially wide-reaching approach which can bridge the gap between agriculture and nutrition. Biofortification can be achieved both by agronomic and genetic approaches. The Importance, genetics and potential of bio-fotification is thoroughly reviewed to provide useful findings for new readers and researchers.

Citation of this article: Gautam, D., Shrestha, B., & Subedi, B. (2021). Bio-fortified maize: Cornerstone in plant breeding to combat hidden hunger in developing countries. *Archives of Agriculture and Environmental Science*, *6*(1), 100-107, https://dx.doi.org/10.26832/24566632.2021.0601013

INTRODUCTION

Maize (*Zea mays*) plays a critical role in meeting the high food demand and is globally one of the most widely cultivated crops as the land area used for maize grain production and also the amount of maize produced per unit area, both have been increasing in recent years (FAOSTAT, 2018). Maize is one of the most valuable and important cereal crops all over the world and together with rice and wheat, provides at least 30% of the food calories to more than 4.5 billion people in 94 developing countries. Maize alone contributes over 20% of food calories in parts of Africa and Mesoamerica (Shiferaw *et al.*, 2011). Malnutrition is estimated to contribute to 45% of all child deaths in developing countries. More than 2 billion people do not get enough essential vitamins and minerals because their diets are not properly balanced which results "Hidden hunger" (Bouis and Saltzman, 2017). The deficiencies of Vitamin A, iron and zinc are the most widespread health problem which causes serious result in higher mortality and morbidity, reduced cognitive abilities and lower work performance (Black, 2003). Biofortification is a feasible, comparatively inexpensive, promising, cost-effective and long-term means of providing micronutrients to individuals of different part of the world through conventional plant breeding or the use of transgenic techniques (Bouis *et al.*, 2011). The potential positive effects of biofortification are obvious: if micronutrient-dense staple crops are widely grown and consumed by the poor, their nutritional status would improve, which could lead to significant health advantages and economic benefits (Zipprian, 1999). Lysine and tryptophan are the building blocks of protein and are also involved as precursors for several neurotransmitters and metabolic regulators (Gupta *et al.*, 2019). Normal maize grain (without biofortification) is poor in these two essential amino acids in the endosperm but QPM possesses nearly two folds higher amount. In the developing countries of Asian regions, Zn deficiency is one of the leading health problems especially in children and women. Among the different interventions developed in the 21st century to overcome malnutrition, biofortification is the most impactful, practical, convenient, sustainable and acceptable intervention. Among the cereal crops, maize is one of the largely grown and consumed in the regions with prevalent Zn malnutrition; therefore, this is a suitable target for Zn biofortification (Maqbool and Beshir, 2019). Based on the factors such as bioavailability ratio (of 12:1), retention up to 50% after storage/ processing, level of nutrients in the host, food matrix and food consumed in the meal, HarvestPlus, a plan of CGIAR (Consultative Group on International Agricultural Research), has fixed a target of 15 µg/g proA per unit of dry weight of maize kernel (Bouis *et al.*, 2011; Pixley, 2013; Owens *et al.*, 2014). Though traditional yellow maize contains high kernel carotenoids, the concentration of provitamin A (proA) is quite low (<2µg/g), compared to the recommended level (15µg/g). It also possesses poor endosperm protein quality due to the low concentration of lysine and tryptophan. A natural variant of crtRB1 (β-carotene hydroxylase) and lcyE (lycopene-ε-cyclase) cause significant enhancement of proA concentration, while recessive allele, opaque2 (o2) enhances the level of these amino acids (Zunjare *et al.*, 2018). In the present context, genetic-engineering based food biofortification is a promising way to address the hidden hunger especially, where breeding is not rewarding due to lack of genetic variability. Genetic modification through gene technology is a swift and accurate method to develop nutrient denser crops without any recurrent investment as compared to different strategies (Kirthee *et al.*, 2019). This can have a varied success rate but it have a bright future to adress the malnutration challage in long run (Garg *et al.*, 2018).

Maize in the global scenario

Maize (*Zea mays ssp. mays*) which belongs to the tribe Maydae of the family Poaceae, was originated in Mexico and Central America (Hossain *et al.*, 2016). Maize (*Zea mays* L.) is one of the oldest florae that humans have domesticated and its success is partly due to its high yield, productivity, and its exceptional geographic adaptability (FAO, 2020). It delivers around 30% of the food calories to 4.5 billion people of 94 developing countries together with other cereals. Global production of maize has got hold of about 1124 million tonnes from 196-million-hectare area distributed in as many as 168 countries with the productivity of 5.71 t ha^{-1}. 1045 million metric tons global production in 2017-18 providing 15-56% of total calorie intake in Sub-Saharan Africa, Latin America, and Asia worldwide due to which it is preferred staple food for 900 million people day (Issa, 2018). Maize comprises approximately 72% starch, 10% protein, and 4% fat, contributing an energy density of 365 Kcal/100 g and is grown throughout the world, with the United States, China, and Brazil being the top three maize-producing countries in the world, producing approximately 563 of the 717 million metric tons/year (Ranum *et al.*, 2014). Maize is generally categorized into one of two broad groups: yellow and white. Yellow maize accounts for the bulk of the total world maize market. It is grown in most northern hemisphere countries and is predominantly used for animal feed. White maize is produced for food in Latin America, Southern Africa, and South Asia under a wide range of climate conditions. Market prices are usually higher for white than for yellow maize because consumers perceive it as a superior-good (FAO, 2020). Global trade of maize in 2019 remained around the average of the previous two years, with larger export from South America, while wheat exports expanded, especially from the European Union, Argentina, and Ukraine (FAO, 2020). Maize production of top maize producing countries can be perceived in Figure 1 (USDA, 2020) Indonesia will become one of the largest maize importers by 2021 (4.3Mmt) (Issa, 2018).

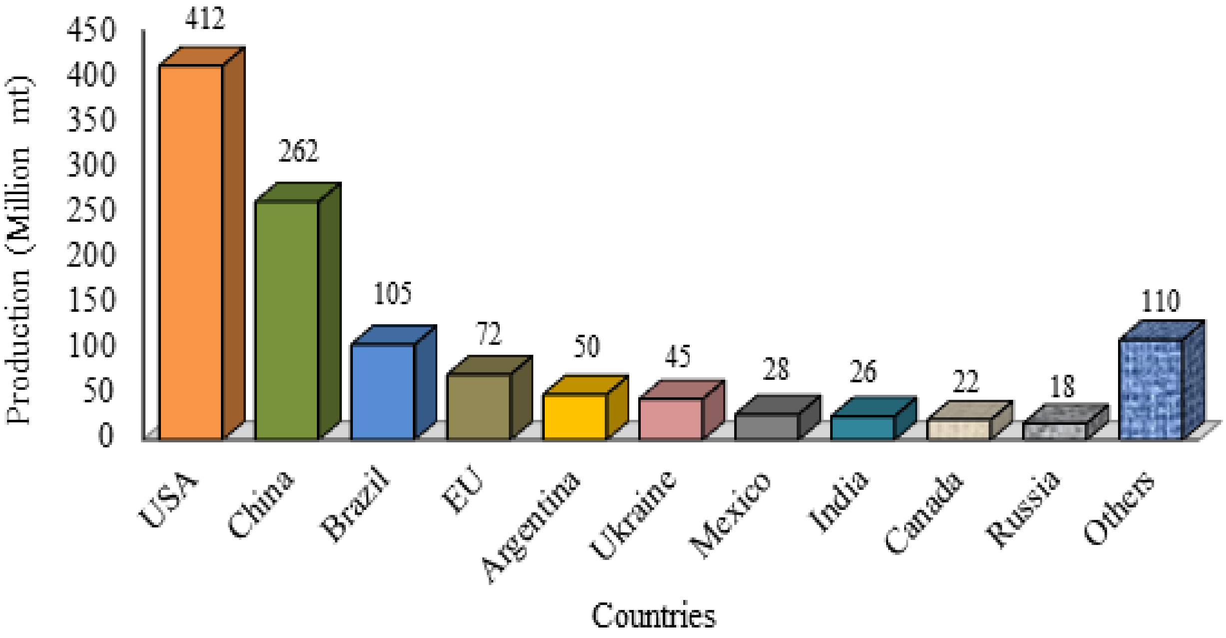


Figure 1. *Maize production by top producing countries in Million metric tons (Source: FAOSTAT, 2018)*

Maize in Nepal

Maize is the second foremost important crop after rice regarding area and production in Nepal (Sapkota and Pokhrel, 2013). In the hilly region of Nepal, it is a way of life and also a traditional crop grown for food, feed and fodder. The demand of maize has been constantly growing by about 5% in the last decades, annually (Sapkota and Pokhrel, 2013). The area and production of maize in 2073/74, 2074/75 and 2075/76 is 924321 ha; 2336675 mt, 954158 ha; 2555847 mt and 956447 ha; 2713635 mt respectively. Maize covers about 81% (Mountain- 10.45% and Hill- 70.23%) of the total cultivated area in the rain-fed ecosystem of the hills and mountain area. (MoAC, 2009). The cultivated area of maize in Terai, Mid Hills and High Hills was 18.47%, 72.96% and 8.57%, respectively. Maize plays a vital role in the livelihood of the people living in the hills and mountains of the country by contributing about 26.8% of the total food requirement among cereals. Maize is grown under rainfed conditions during the summer season (April-August) as a single crop or relayed with millet later in the season. Maize can also be grown in the winter and spring with irrigation in the Terai, Inner-Terai, valleys, and low-lying river basin areas. More than two-third of the maize is used for direct human consumption at the farm level which is produced in the Mid Hills and High Hills. The ratio of human consumption to total production is higher in less accessible areas. In Terai, less than 50% of the maize is used for human consumption and a major part of the production goes to the market (Paudyal, 2001). Maize contributes about 6.88 % of the total agricultural GDP supplied to the nation (MoAC, 2008). The national maize production quantity from 2008 to 2018 is shown in Figure 2. In 2019, maize production for Nepal was 2,550 thousand tonnes. Maize production of Nepal increased from 833 thousand tonnes in 1970 to 2,550 thousand tonnes in 2019 growing at an average annual rate of 2.64% (USDA, 2020).

Importance of maize

Maize has emerged as a crop of global importance due to its multiple ends uses can be processed into a variety of human food and industrial goods, including starch, inducements, oil, brews, glue, industrial liquor, and fuel ethanol (Ranum *et al.*, 2014). Moreover, maize serves as a model organism for biological research worldwide. It is used for making edible oil and is also a significant source of biofuel production in the world (Nayava, 2010). In the last 10 years, the use of maize for fuel production significantly increased, accounting for approximately 40% of the maize production in the United States. As the ethanol industry absorbs a larger share of the maize crop, higher maize prices will strengthen demand competition and could affect maize prices for animal and human ingestion. Low production costs, sideways with the high consumption of maize flour and cornmeal, particularly where micronutrient deficiencies are mutual public health teething troubles, make this food staple an ideal food vehicle for fortification (Ranum *et al.*, 2014). Maize is responsible for over 20 % of total calories in human diets in 21 countries and over 30 % in 12 countries that are home to a total of more than 310 million people (Shiferaw *et al.*, 2011). At present, the developed world uses more maize than the developing world, but forecasts indicate that by the year 2050, the demand for maize in the developing countries will duple owing to the rapid growth in the poultry industry, the biggest driver of growth in maize production (Rosegrant *et al.*, 2009; Prasanna, 2014). The cost-reducing effects of using Quality Protein Maize (QPM) in the poultry feed industry in Nepal has been accessed (Thapa *et al.*, 2020).

Figure 2. *Trend of Maize production in Nepal (Source: FAOSTAT, 2018).*

Bio-fortification

A process of improving the concentration of minerals and vitamins in food staples eaten usually by the poor may be either through conventional plant breeding or through the procedure of transgenic methods is known as bio-fortification (Bouis *et al.*, 2011). Over the past 15 years, research has demonstrated that another strategy, called bio-fortification, is an effective complement to these approaches in addressing micronutrient deficiency and related health problems (Saltzman *et al.*, 2017). The bio-fortification tactic pursues to take advantage of the consistent daily consumption of large quantities of food staples by all family members, including women and children, who are most at risk for micronutrient undernourishment (Bouis *et al.*, 2011). Bio-fortification can improve the nutritional content of the staple foods poor people already eat, providing a comparatively inexpensive, cost-effective, sustainable, long-term means of delivering more micronutrients to the poor (Kumar *et al.*, 2019). This approach not only will lower the number of severely undernourished people who require treatment by balancing interventions, but also will help them maintain improved nutritional status. Moreover, biofortification provides a feasible means of reaching malnourished rural populations who may have limited access to commercially promoted fortified foods and supplements (Bouis *et al.*, 2011). Bio-fortification differs from ordinary fortification because it focuses on making plant foods more nutritious as the plants are growing, rather than having nutrients added to the foods when they are being processed. The biofortified seeds can be easily reproduced by poor farmers, and thus the seeds are a sustainable means to target remote rural communities not served by conventional seed markets (Qaim *et al.*, 2007).

Importance of pro-vitamin A bio-fortified maize

Bio-fortified maize contains enhanced provitamin A concentrations and has been bio efficacious in animal and small human studies (Gannon *et al.*, 2014). The main objective of pro-vitamin A biofortified maize breeding project high nutrient density must be combined with high yields and high profitability with demonstrable efficacy in reducing VAD which is acceptable to consumers (Bouis and Welch, 2014). Vitamin A deficiency is widely prevailing in children and women of developing countries. Lack of vitamin A causes night blindness, growth hindrance, xerophthalmia, and increases the proneness against epidemic diseases (Maqbool *et al.*, 2018). Pro-vitamin A biofortified maize may contribute to alleviating vitamin A deficiency (VAD), in developing countries (Azmach *et al.*, 2013). However, processing the maize into food products may reduce its provitamin A content (Kirthee *et al.*, 2013).

Importance of quality protein maize in nutrition security

Dietary protein and amino acid requirement recommendations for normal “healthy” children and adults have varied considerably with 2007 FAO/WHO protein requirement estimates for children lower, but dietary essential AA requirements for adults more than doubled (Ghosh *et al.*, 2012). Biofortified crops, bred for improved nutritional excellence, can alleviate nutritional deficiencies if they are produced and consumed in sufficient quantities. Under nutrition is a persistent problem in Africa, exclusively in rural areas where the poor largely depend on staples and have partial access to a diverse diet. Quality protein maize (QPM) consists of maize varieties biofortified with increased lysine and tryptophan levels. Several studies in controlled settings have indicated the positive impact of QPM on the nutritional status of children (Akalu *et al.*, 2010). Under nutrition was pervasive, and maize was the dominant food in the children’s complementary diets. Those major maize producing and consuming areas of Africa, home cultivation and use of QPM in children’s diets could reduce or avert growth faltering and may in some cases support catch-up growth in weight (Akalu *et al.*, 2010). To combat protein-energy imbalance, plant breeders have developed quality protein maize (QPM) genotypes by using recessive opaque2 (o2) allele (Mertz *et al.*, 1964) in conjunction with endosperm modifiers at CIMMYT, Mexico (Vasal *et al.*, 1980).

Breeding for quality protein maize (Zn, protein vitamin enriched)

Quality protein maize (QPM) was the first biofortified crop possessing balanced protein having higher lysine and tryptophan which has been distributed in Africa using both methods: conventional breeding and transgenic techniques (De groote *et al.*, 2010). Different breeding strategies including diversity analysis, introduction and stability analysis of exotic germplasm, hybridization, heterosis breeding, mutagenesis and marker-assisted selection are practiced for exploring maize germplasm and development of pro-vitamin A enriched cultivars (Maqbool *et al.*, 2018). Genome-wide association variety and development of transgenic maize genotypes are also being practiced, whereas RNA interference and genome editing tools could also be used as potential strategies for provitamin A biofortification of maize genotypes (Shrestha *et al.*, 2016). The use of these breeding strategies for provitamin A biofortification of maize is comprehensively reviewed to provide a working outline for maize breeders (Maqbool *et al.*, 2018) The biofortified maize varieties were better sources of most of the essential amino acids comparative to the white variety, but, similar to the white maize, they were deficient in histidine and lysine (Kirthee *et al.*, 2013). HarvestPlus seek out to improve and allocate varieties of food staples (paddy, wheat, maize, cassava, pearl millet, beans, and sweet potato) that are high in iron, zinc, and provitamin A through an interdisciplinary, international alliance of scientific and technical institutions and implementing assistances in developing and developed nations (Bouis *et al.*, 2011).

The quality of the grains of the biofortified maize varieties was superior to that of the white maize grain, although, the biofortified grains were more prone to fungal attack, emphasizing a need to combine the superior nourishing traits of provitamin A- biofortified maize with desirable grain quality, especially resistance to fungal infection, in a breeding program (Kirthee *et al.*, 2013).

Zn biofortification of maize could be achieved through agronomic and genetic approaches (Maqbool and Beshir, 2019). Zn bioavailability could be increased by reducing the antinutritional dynamics or by increasing the bioavailability enhancers. Kernel Zn concentration could be improved through hybridization and selections, whereas genetically engineered tries for improving Zn uptake from soil, loading in xylem, remobilization in grains and sequestration in endosperm can additionally improve the kernel Zn concentration (Maqbool and Beshir, 2019). (De groote *et al*., 2014) concluded that QPM may prefer for its sensory characteristics or, at least, as well acknowledged as conventional maize. Information on nutritional benefits increases rural consumers' willingness to pay for it, so information campaigns may be effective in increasing demand for QPM varieties. However, these varieties need to be competitive for other characteristics that rural households value, in particular, field and storage qualities. Vivek Maize Hybrid-9 (VH-9), a popular single-cross hybrid developed by crossing CM 212 and CM 145 was released for commercial cultivation in India showed 41% increase in tryptophan and 30% increase in lysine over the original hybrid. The grain yield of the improved hybrid was on par with the original hybrid (Gupta *et al*., 2013). Using adaptable low land tropical germplasm with downy mildew and stalk rot, background can be improved disease resistance of susceptible populations, both in normal maize and QPM (Denic *et al*., 2007). In Nepal, six genotypes of quality protein maize was evaluated, out of which the better genotypes concerning grain yield and location were Poshilo Makai-1 and Farmer's Variety for Doti and Rampur whereas SO3TLYQ-AB-01 and S99TLYQ- B for Surkhet. Correspondingly SO3TLYQ-AB-01 was superior for Pakhribas, SOTLYQ-AB-02 for Lumle and Poshilo Makai-1 and S99TLYQ-B for Kabre condition. Therefore these varieties concerning their specific adaptation can be suggested for common cultivation.

Genetic variation in maize and genetic makeup of biofortified maize

Several carotinoids present in maize are very important for human health. Scientists have opened about the varieties of maize that have naturally high levels of provitamin A (Garg *et al*., 2018). Maize shows considerable natural variation for kernel carotenoids, with some of the genotypes containing as high as 66.0 µg/g which includes α and β-carotene and β- cryptoxanthin as a vitamin A precursors which are very essential for different system in the human body and for the prevention of diet related chronic dieaseses (Harjes *et al*., 2008; Pillay *et al*., 2011; Pixley, 2013). In yellow maize kernel, comprising two carotenes, and also three xanthophylls, β-cryptoxanthin, zeaxanthin, and lutein carotenoids are present (Weber, 1987). In general, provitamin A carotenoids constitute 10–20 % of total carotenoids in maize with zeaxanthin and lutein each commonly represent 30–50 % wheras the quantities of provitamin A in traditional yellow maize varieties kernel range from 0.25µg to 2.5µg/g dry weight. The concentrations of α-carotene, β-carotene, and β-cryptoxanthin, range from 0 to 1.3, 0.13 to 2.7, and 0.13 to 1.9 nmol/g, respectively in a typical maize (Kurilich and Juvik, 1999). Although β-carotene has the highest provitamin A activity, it is present in a relatively low concentration (0.5–1.5 µg/g) in most yellow maize grown and consumed throughout the world (Harjes *et al*., 2008).

To alleviate the vitamin A deficiency from rural areas of developing countries, biofortification of maize of respective regions with provitamin A carotenoids is the only feasible way, since this has been ensured for the better compliance and target (Watson, 1962). Based on the factors such as bioavailability ratio (of 12:1), retention up to 50% after storage/processing, level of nutrients in the host, food matrix and food consumed in the meal, HarvestPlus, a plan of CGIAR (Consultative Group on International Agricultural Research) Nutritionists have estimated that 15 µg provitamin A per gram dry weight of kernel could greatly alleviate vitamin A deficiency (www.harvestplus.org). To meet this target, researchers have been pursuing development of proA-rich maize hybrids through different approaches of genetic enhancement. In general, tropical maize contains more β -cyrptoxanthin and less β-carotene then temperate maize and because the emphasis was on enhancing β-carotene concentration, more of the initial breeding source of high provitamin A germplasm were selected from temperate regions. Subsequently, genetic assiociation mapping studies using three diverse maize germplasm panels, selected to emcompass a wide range of carotinoid contents and ratios, have identified favourable alleles of genetic encoding two key enzymes in carotinoid biosynthetic pathway. Enhancement in proA in maize can be done by favorable alleles of lycopene ε-cyclase (lcyE), phytoene synthase (PSY) and β-carotene hydroxylase1 (crtRB1) genes (Harjes *et al*., 2007; Yan *et al*., 2010; Babu *et al*., 2013). Germplasm carrying the favourable crtRB1 allele in homozygous form has been identified with β-carotene concentation up to 26µg/g DW and total provitamin A as high as 30µg/g DW which are currently being used in different breeding programmes.

The availability of a well characterized biosynthetic pathway facilitated the identification of genes controlling critical steps in the carotenoid biosynthetic pathway in maize (Harjes *et al*., 2008; Yan *et al*., 2010). The breeding strategy involved selection for increased flux into the carotenoid pathway at PSY, and reducing flux into the α branch towards lutein, which has little or no proVA activity, and more into the β side towards β carotene and β cryptoxanthin, which have proVA activity (Harjes *et al*., 2008; Yan *et al*., 2010; Chandler *et al*., 2013). Selection for the favorable allele at the CRTRB1 locus has resulted in four times higher proVA content than that resulting from the wild type allele (Yan *et al*., 2010; Babu *et al*., 2013). Genetic variation at key loci has been exploited through breeding to create sufficient diversity, to enable long term genetic gain through selection, and reach target levels deemed adequate to impact human nutrition (Dhliwayo *et al*., 2014; Suwarno *et al*., 2014).

Breeding techniques for provitamin A biofortified maize

Exploiting the existing natural genetic variability for provitamin A carotenoids, maize breeders have succeeded in developing =< -15 µg β-carotene/g dry kernel weight (Yan *et al*., 2010). The less complex nature of control of provitamin A content, high heritability, mode of inheritance regulated primarily by additive genetic effects, and the statistically non-significant correlation between PVA and agronomic performance suggested that concurrent improvements of PVA carotenoids and grain yield would be possible (Suwarno *et al*., 2014; Menkir *et al*., 2018). Considerable efforts have been made to increase the concentrations of PVA carotenoids in maize through conventional and non-conventional breeding i.e. molecular marker-assisted breeding (Pixley, 2013). Practically no distinction could be made between conventional and non-conventional breeding strategies because these are used interdependently and integratedly *viz*., marker-assisted backcrossing is the integration of conventional hybridization and non-conventional molecular markers. By definition, conventional strategies involve breeding methods without the use of recombinant DNA technology. However, forced hybridization breeding methods which involve the wild relatives and accelerated mutations methods are also included under conventional breeding strategies (Priya *et al*., 2013). Conventional strategies also permit the manipulation of molecular markers to accelerate and precise the breeding efforts. The prevalence of natural or induced variation triggers the manipulation of convenient selection tools for nutritional improvement of maize. Non-conventional strategies involve the manipulations of molecular studies for the genetic improvement of crop plants (Maqbool *et al*., 2018).

DNA markers are derived from molecular genetics and genomic studies, and confer a great promise to conventional plant breeding. The utilization of DNA markers for plant breeding is known as marker-assisted selection (MAS) which greatly improves the precision and efficacy of breeding *(Maqbool et al*., 2018). Studies also proved that colour quantification is not a clear indication of b-carotene quantification (Harjes *et al*., 2008); therefore, the use of MAS holds the great practical potential for provitamin A breeding of maize. MAS facilitates the selection of targeted genes, shortening the duration of varietal development, introgression of desirable trait into agronomically superior variety and improvement of nutritional quality in maize (Babu *et al*., 2013; Prasanna *et al*., 2014). Different germplasms have favorable haplotype with modest frequency differences which facilitate the selection of donor parents from widely adapted resources. MAS for this locus could be used as the alternative for the phenotypic selection or color scoring because the phenotypic selection is unable to differentiate the carotenoid composition. Here, PCR-based diversity analysis of provitamin A carotenoids is more economical than HPLC analysis of carotenoids, thus it is more feasible and accessible to exploit MAS in developing countries.

Hence, molecular markers are widely being used for identifying provitamin A-enriched parental lines, tracking provitamin A-linked alleles during backcross breeding, and expression quantification of pro-vitamin A alleles, (Harjes *et al*., 2008). Molecular marker-assisted selection methods are particularly useful for improving nutritional traits since conventional breeding methods are relatively constrained by the cost and throughout nutritional trait phenotyping (Prasanna *et al*., 2020). Marker-assisted selection for the desired alleles of key proVA genes accelerated genetic gain and allowed to double, sometimes triple, the total concentration of proVA (Harjes *et al*., 2007; Yan *et al*., 2010). The inbreds with elevated lysine, tryptophan, and proA concentration can be used for the development of nutrient-rich maize cultivars in prospect as potential donors and the biofortified maize hybrids developed by using marker-assisted stacking of o2, crtRB1, and lcyE which is enriched with proA, lysine and tryptophan hold greater potential to consecutively alleviate protein-energy malnutrition and vitamin A deficiency across the globe (Zunjare *et al*., 2018). HarvestPlus is using these lines to breed high-yielding varieties of biofortified maize with higher levels of provitamin A to combat vitamin A deficiency. ProVA donors were crossed with elite germplasm of white maize that had high yield potential and good agronomic traits, such as disease resistance and drought tolerance (Kondwakwenda, 2018).

Breeding for zinc bio-fortified maize

Zn biofortification of maize could be achieved through agronomic and genetic approaches. Discussion of agronomic approaches with genetic approaches is a prerequisite because soils in developing countries are deficit of Zn and availability of Zn in soils is mandatory for estimating the genetic responses of maize genotypes through genetic approaches (Maqbool and Beshir, 2019). Zn bioavailability could be increased by reducing the antinutritional factors or by increasing the bioavailability enhancers. Kernel Zn concentration could be improved through hybridization and selections, whereas genetically engineered attempts for improving Zn uptake from the soil, loading in xylem, remobilization in grains and sequestration in endosperm can further improve the kernel Zn concentration (Maqbool and Beshir, 2019).

Conclusion

Challenges and evolution in agriculture and food production are two sides of a single coin. Vitamin A deficiency is a widely prevalent health problem that is affecting children and women in developing countries. To cope with this challenge, we needed a large boost strategy. Bio-fortified staple crops have turned out to be the most effective way of supplying higher quality proteins as well as micronutrients. Research conducted during the last 15 years has led to the progress and release of a large number of bio-fortified varieties of various food crops in general and also in particular cereals. The combinations of nutritional quality traits, including QPM, PVA, high-Zn, etc. in both maize grain and in fresh corn has consumer appeal, and contributes to national initiatives and sustainable development goals for enhancing nutrition. The rapid advances that have been made in understanding the genetic control of many macro and micronutrients in maize grains, together with the availability of new tools/ technologies such as genomic selection, will accelerate the rate of genetic gain for improved nutrient content in maize. Advances in phenotyping coupled with molecular breeding facilitated the achievement of the breeding targets for various nutrients in maize. Conclusively, after the huge success of QPM in Sub-Saharan Africa, bio-fortified yellow kernel maize rich in vitamin A was adopted by the farmers as well as consumers resulting in acceptance of pro-vitamin A rich maize hybrids/ composites/synthetics. Marker-assisted selection is a new avenue for quicker genetic improvement of traits and to abridge the duration of variety development. In the coming years, bio-fortification is expected to be increasingly integrated into international and national crop development programs, crop and food value chains, and national policies and standards. This review articulates that the adoption of amenable strategy mutual with improved cultivation practices can be the only upcoming pathway for human welfare.

ACKNOWLEDGEMENT

The author's express sincere thanks and gratefully acknowledge all helping hands of this study. All personages who provided valuable feedback and suggestions on this paper are also appreciated.

REFERENCES

Akalu, G., Taffesse, S., Gunaratna, N. S., & De Groote, H. (2010). The effectiveness of quality protein maize in improving the nutritional status of young children in the Ethiopian highlands. *Food and Nutrition Bulletin*, *31*(3), 418–430, https://doi.org/10.1177/156482651003100304

Azmach, G., Gedil, M., Menkir, A., & Spillane, C. (2013). Marker-trait association analysis of functional gene markers for provitamin A levels across diverse tropical yellow maize inbred lines. *BMC Plant Biology*, *13*(1), 227.

Babu, R., Rojas, N. P., Gao, S., Yan, J., & Pixley, K. (2013). Validation of the effects of molecular marker polymorphisms in LcyE and CrtRB1 on provitamin A concentrations for 26 tropical maize populations. *Theoretical and Applied Genetics*, *126*(2), 389–399, https://doi.org/10.1007/s00122-012-1987-3

Black, M. M. (2003). Micronutrient deficiencies and cognitive functioning. *The Journal of Nutrition*, *133*(11), 3927S-3931S.

Bouis, H. E., & Saltzman, A. (2017). Improving nutrition through biofortification: a review of evidence from HarvestPlus, 2003 through 2016. *Global food Security*, *12*, 49-58.

Bouis, H. E., & Welch, R. M. (2010). Biofortification—a sustainable agricultural strategy for reducing micronutrient malnutrition in the global south. *Crop Science*, 50, S-20.

Bouis, H. E., Hotz, C., McClafferty, B., Meenakshi, J. V., & Pfeiffer, W. H. (2011). Biofortification: A new tool to reduce micronutrient malnutrition. *Food and Nutrition Bulletin*, *32*(1_suppl 1, S31-S40, https://doi.org/10.1177/15648265110321s105

Chandler, K., Lipka, A. E., Owens, B. F., Li, H., Buckler, E. S., Rocheford, T., & Gore, M. A. (2013). Genetic analysis of visually scored orange kernel color in maize. *Crop Science*, *53*(1), 189-200.

De Groote, H., Gunaratna, N. S., Ergano, K., & Friesen, D. (2010). Extension and adoption of biofortified crops: Quality protein maize in East Africa (No. 308-2016-5035).

De Groote, Hugo, Chege, C. K., Tomlins, K., & Gunaratna, N. S. (2014). Combining experimental auctions with a modified home-use test to assess rural consumers' acceptance of quality protein maize, a biofortified crop. *Food Quality and Preference*, *38*, 1-13, https://doi.org/10.1016/j.foodqual.2014.04.014

Denic, M., Chauque, P., Fato, P., Senete, C., Mariote, D., & Haag, W. (2007). Breeding approaches in simultaneous selection for multiple stress tolerance of maize in tropical environments. *Genetika*, *39*(2), 113–124, https://doi.org/10.2298/gensr0702113d

Dhliwayo, T., Palacios Rojas, N., Crossa, J., & Pixley, K. V. (2014). Effects of S1 recurrent selection for provitamin A carotenoid content for three open pollinated maize cultivars. *Crop Science*, *54*(6), 2449-2460.

FAO (Food and Agricultural Organization of the United Nations), 2020. Statistical database. (accessed 2020-07-18).

FAOSTAT. (2013). Agricultural statistics database. Rome: World Agricultural. Information Center. Disponível em< http://faostat. Fao . org/site/567/ DesktopDefault. Aspx.

FAOSTAT. (2018). Available online: http://www. fao. org/faostat/en/# data. QC (accessed on January 20 Faostat, F. A. O. (2018). Available online: http://www. fao. org/faostat/en/# data. QC (accessed on January 2019).18).

FAOSTAT. (2018). Retrieved 02,24,2020, from Statistics Division, Food and Agriculture Organization of the United Nations. www.fao.org/faostat/en/#data.

Gannon, B., Kaliwile, C., Arscott, S. A., Schmaelzle, S., Chileshe, J., Kalungwana, N., & Tanumihardjo, S. A. (2014). Biofortified orange maize is as efficacious as a vitamin A supplement in Zambian children even in the presence of high liver reserves of vitamin A: a community-based, randomized placebo-controlled trial. *The American Journal of Clinical Nutrition*, *100*(6), 1541-1550, https://doi.org/10.3945/ajcn.114.087379.Ensuring

Garg, M., Sharma, N., Sharma, S., Kapoor, P., Kumar, A., Chunduri, V., & Arora, P. (2018). Biofortified crops generated by breeding, agronomy, and transgenic approaches are improving lives of millions of people around the world. *Frontiers in Nutrition*, 5, 12.

Ghosh, S., Suri, D., & Uauy, R. (2012). Assessment of protein adequacy in developing countries: quality matters. *British Journal of Nutrition*, 108(S2), S77-S87. https://doi.org/10.1017/S0007114512002577

Gupta, H. S., Hossain, F., & Muthusamy, V. (2015). Biofortification of maize: An Indian perspective. Indian J. Genet, 75(1), 1-22.

Gupta, H. S., Hossain, F., Muthusamy, V., & Zunjare, R. U. (2019). Marker-assisted breeding for enrichment of provitamin A in maize. In Quality breeding in field crops, Springer, Cham. pp. 139-157

Gupta, H. S., Raman, B., Agrawal, P. K., Mahajan, V., Hossain, F., & Thirunavukkarasu, N. (2013). Accelerated development of quality protein maize hybrid through marker-assisted introgression of opaque-2 allele. *Plant Breeding*, *132*(1), 77–82, https://doi.org/10.1111/pbr.12009

Harjes, C. E., Rocheford, T. R., Bai, L., Brutnell, T. P., Kandianis, C. B., Sowinski, S. G., & Yan, J. (2008). Natural genetic variation in lycopene epsilon cyclase tapped for maize biofortification. Science, 319(5861), 330-333.

Hossain, F., Muthusamy, V., Bhat, J. S., Jha, S. K., Zunjare, R., Das, A., & Kumar, R. (2016). Maize. In Broadening the Genetic Base of Grain Cereals (pp. 67-88). Springer, New Delhi. https://doi.org/10.1007/978-81-322-3613-9

Issa, A. (2018). CIMMYT' s Maize Innovations and Interventions in South Asia. 34, [Presentation]. https://repository.cimmyt.org/bitstream/handle/10883/20021/60148.pdf?sequence=1

Kirthee, P., Muthulisi, S., John, D., & Frederick, J. V. (2013). Influence of biofortification with provitamin A on protein, selected micronutrient composition and grain quality of maize. *African Journal of Biotechnology*, *12*(34), 5285–5293, https://doi.org/10.5897/ajb12.2920

Kondwakwenda, A. K. (2018). Provitamin A maize biofortification in sub-Saharan Africa. *Maydica*, *63*(3), 9.

Kumar, S., Palve, A., Joshi, C., Srivastava, R. K., & Rukhsar. (2019). Crop biofortification for iron (Fe), zinc (Zn) and vitamin A with transgenic approaches. *Heliyon*, *5*(6), e01914, https://doi.org/10.1016/j.heliyon.2019.e01914

Kurilich, A. C., & Juvik, J. A. (1999). Quantification of Carotenoid and Tocopherol Antioxidants in Zea mays. *Journal of Agricultural and Food Chemistry*, *47*(5), 1948-1955.

Maqbool, M. A., & Beshir, A. R. (2019). Zinc biofortification of maize (Zea mays L.): Status and challenges. *Plant Breeding*, 138(1), 1–28, https://doi.org/10.1111/pbr.12658

Maqbool, M. A., Khan, S., Aslam, M., & Beshir, A. (2018). Breeding for provitamin A biofortification of maize (*Zea mays* L.). 451–469, https://doi.org/10.1111/pbr.12618

Menkir, A., Palacios-Rojas, N., Alamu, O., Dias Paes, M. C., Dhliwayo, T., Maziya-Dixon, B., & Rocheford, T. (2018). Vitamin A-biofortified maize: exploiting native genetic variation for nutrient enrichment (No. 2187-2019-667).

Mertz, E. T., Bates, L. S., & Nelson, O. E. (1964). Mutant gene that changes protein composition and increases lysine content of maize endosperm. *Science*, *145* (3629), 279-280, https://www.fas.usda.gov/commodities/corn

MoAC (2005): Statistical information on Nepalese agriculture. Agri-business promotion and statistic division, Ministry of Agriculture and Co-operatives, Singha Darbar, Kathmandu, Nepal.

MoAC (2013). Statistical Information on Nepalese Agriculture. Government of Nepal Ministry of Agricultural Development Agribusiness Promotion and Statistics Division Agri Statistics section Singha Durbar, Kathmandu Nepal. 2013.

MoAC (Ministry of agriculture and Cooperatives). 2008. Selected indicators of Nepalese agriculture and population. Agribusiness promotion and statistical division/Gender equity and environment division, Ministry of Agriculture and Cooperatives (MOAC), Singha Durbar, Kathmandu, Nepal.

MoAC (Ministry of Agriculture and Cooperatives).. 2009. Statistical information on Nepalese agriculture (2008/09), Agribusiness promotion and statistical division, Ministry of Agriculture and Cooperatives (MOAC), Singha Durbar, Kathmandu, Nepal available on http://www.moac.gov.np/publications/statistics/new/New%20Cereal%20Crops.pdf.

Nayava, J. L. (2010). Impact of climate change on production and productivity: A case study of maize research and development in Nepal. *Journal of Agriculture and Environment*, *11*, 59-69, https://doi.org/10.3126/aej.v11i0.3653

Owens, B. F., Lipka, A. E., Magallanes-Lundback, M., Tiede, T., Diepenbrock, C. H., Kandianis, C. B., & Buckler, E.S. (2014). A foundation for provitamin A biofortification of maize: genome-wide association and genomic prediction models of carotenoid levels. *Genetics*, *198*(4), 1699-1716, https://doi.org/10.1534/genetics.114.169979

Paudyal, K. R. (2001). Maize in Nepal: production systems, constraints, and priorities for research. CIMMYT.

Pillay, K., Derera, J., Siwela, M., & Veldman, F. J. (2011). Consumer acceptance of yellow, provitamin A-biofortified maize in KwaZulu-Natal. *South African Journal of Clinical Nutrition*, *24*(4), 186-191, https://doi.org/10.1080/16070658.2011.11734386

Pillay, K., Siwela, M., Derera, J., & Veldman, F. J. (2014). Provitamin A carotenoids in biofortified maize and their retention during processing and preparation of South African maize foods. Journal of food science and technology, *51*(4), 634-644, https://doi.org/10.1007/s13197-011-0559-x

Pixley, K. V., Palacios-Rojas, N., Babu, R., Mutale, R., Surles, R., & Simpungwe, E. (2013). Biofortification of maize with provitamin A carotenoids In: Tanumihardjo SA editor. Carotenoids in human health. New York: Springer Science and Business Media; pp. 271-92, https://doi.org/10.1007/978-1-62703-203-2

Prasanna, B M, Vivek, B., Sadananda, A. R., Jeffers, D. P., Zaidi, P. H., Boeber, C., Erenstein, O., Babu, R., Nair, S. K., & Gerard, B. (2014). 12th Asian Maize Conference and Expert Consultation on maize for food, feed, nutrition; and environmental security; Bangkok (Thailand), 30-1 Aug-Nov 2014: extended summaries. November.

Prasanna, Boddupalli M., Palacios-Rojas, N., Hossain, F., Muthusamy, V., Menkir, A., Dhliwayo, T., Ndhlela, T., San Vicente, F., Nair, S. K., Vivek, B. S., Zhang, X., Olsen, M., & Fan, X. (2020). Molecular Breeding for Nutritionally Enriched Maize: Status and Prospects. Frontiers in Genetics, 10(February), 1–16. https://doi.org/10.3389/fgene.2019.01392

Priya, S., Anjana, P., & Major, S. (2013). Identification of the RAPD Marker linked to Powdery Mildew Resistant Gene (s) in Black Gram by using Bulk Segregant Analysis. *Research Journal of Biotechnology*, *8*(2), 9-15.

Qaim, M., Stein, A. J., & Meenakshi, J. V. (2007). Economics of biofortification. *Agricultural Economics*, *37*, 119-133.

Qaim, M., Stein, A. J., & Meenakshi, J. V. (2007). Economics of biofortification. *Agricultural Economics*, 37, 119-133, https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1574-0862.2007.00239.x

Ranum, P., PeñaRosas, J. P., & GarciaCasal, M. N. (2014). Global maize production, utilization, and consumption. Annals of the new York academy of sciences, *1312*(1),105-112, https://doi.org/10.1111/nyas.12396

Rosegrant, M. R., Ringler, C., Sulser, T. B., Ewing, M., Palazzo, A., Zhu, T., & Batka, M. (2009). Agriculture and food security under global change: Prospects for 2025/2050. International Food Policy Research Institute, Washington, DC, 145-178.

Saltzman, A., Birol, E., Oparinde, A., Andersson, M. S., AsareMarfo, D., Diressie, M. T., & Zeller, M. (2017). Availability, production, and consumption of crops biofortified by plant breeding: current evidence and future potential. *Annals of the New York Academy of Sciences*, *1390*(1), 104-114, https://doi.org/10.1111/nyas.13314

Sapkota, D., & Pokhrel, S. (2013). Community based maize seed production in the hills and mountains of Nepal: A review. *Agronomy Journal of Nepal*, *1*, 107–112, https://doi.org/10.3126/ajn.v1i0.7550

Shiferaw, B., Prasanna, B. M., Hellin, J., & Bänziger, M. (2011). Crops that feed the world 6. Past successes and future challenges to the role played by maize in global food security. *Food Security*, *3*(3), 307, https://doi.org/10.1007/s12571-011-0140-5

Shrestha, J., Kunwar, C. B., Upadhyaya, J., Giri, M., Katuwal, R. B., Acharya, R., Bahadur Gurung, S., Adhikari, B. N., Paudel, A. P., & Paneru, R. B. (2016). Genotype × environment interactin of quality protein maize grain yield in Nepal. *Journal of Maize Research and Development*, 66–73.

Suwarno, W. B., Pixley, K. V., PalaciosRojas, N., Kaeppler, S. M., & Babu, R. (2014). Formation of heterotic groups and understanding genetic effects in a provitamin A biofortified maize breeding program. *Crop Science*, *54*(1), 14-24.

Thapa, G., Gautam, S., Rahut, D. B., & Choudhary, D. (2020). Cost advantage of biofortified maize for the poultry feed industry and its implications for value chain actors in Nepal. *Journal of International Food & Agribusiness Marketing*, 1 -25, https://doi.org/10.1080/08974438.2020.1780179

USDA. (2020). Wheat export by countries. http://www.worldstopexports.com/wheat-imports-by-country. https://knoema.com/atlas/Nepal/topics/Agriculture/Crops-Production-Quantity-tonnes/Maize-production

Vasal, S. K., Villegas, E., Bjarnason, M., Gelaw, B., & Goertz, P. (1980). Genetic modifiers and breeding strategies in developing hard endosperm opaque-2 materials. Genetic modifiers and breeding strategies in developing hard endosperm opaque-2 materials., 37-73.

Watson, S. A. (1962). The yellow carotenoid pigments of corn. In 17th Hybrid Corn Industry Res Conf. American Seed Trade Association, Chicago, Ill (pp. 92-100).

Weber, E. J. (1987). Carotenoids and tocols of corn grain determined by HPLC. *Journal of the American Oil Chemists' Society*, 64(8), 1129-1134, http://www.fao.org/faostat/en/#data/QC.

Yan, J., Kandianis, C. B., Harjes, C. E., Bai, L., Kim, E. H., Yang, X., & Fernandez, M. G. S. (2010). Rare genetic variation at Zea mays crtRB1 increases β-carotene in maize grain. *Nature Genetics*, *42*(4), 322-327, https://doi.org/10.1038/ng.551

Zipprian, C. (1999). Economics of SR. NACE - International Corrosion Conference Series, 1999-April.

Zunjare, R. U., Hossain, F., Muthusamy, V., Baveja, A., Chauhan, H. S., Bhat, J. S., & Gupta, H. S. (2018). Development of biofortified maize hybrids through marker-assisted stacking of β-carotene hydroxylase, lycopene-ε-cyclase and opaque2 genes. *Frontiers in Plant Science*, *9*, 178, https://doi.org/10.3389/fpls.2018.00178