

Biofortification: A New Approach to Enhance the Nutrition in Maize Crop

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Abstract:

Approximately, 3 billion people are suffering from micronutrient deficiency worldwide. The deficiencies of micronutrients in maize crops ensure irregular diets are common among a large population. Biofortification, the mean of rearing nutrients into food crops, administers the sustainability and deep-rooted strategy to distribute micronutrients to agrarian populations in growing countries. The biofortification strategy of maize crops has focused on three ambiguous micronutrients such as vitamin A, Iron, and especially zinc to increase nutrition level. The concentration of nutrients in maize crops can be increased by using genetic manipulation, conventional breeding, and agronomic biofortification. Biofortification is being resulted to produce nutrients at a large-scale and enhance yields, concentrations, and nutrition levels of targeted nutrients in maize crops. To accelerate the development of biofortification techniques is essential to enhance the availability of staple crops to overcome the malnutrition in a population. This review highlights the advancements to enhance maize crop nutrition and analyses the challenges faced in malnutrition.

Keywords: Biofortification, Micronutrients, Genetic Manipulation, Conventional Breeding, Agronomic approaches, Malnutrition

1. Introduction:

Zea mays L. is one of the precondition cereal byproducts in nature both in the process of feed and food and is a constituent of the Poaceae family and commonly cultivated in the autumn and spring seasons. Comprehensively, its world population depends upon wheat and rice, ranking

third and acknowledge a predominant food in many communities, specifically in the tropic and sub-tropic regions (Mohammadi et al., 2017). In Pakistan, the large-scale producers of maize crops are in the Punjab and KPK, computing for around 97% of absolute production. (Tariq and Iqbal, 2010). Sugar-rich varieties are normally developed for natural utilization, while varieties of cornfield are recycled for an animal meal and chemical feedstock processes. In addition, corn is also a significant source of gluten, starch, and oil, which can be broken down and energetically discarded to make high fructose corn syrup. Corn provides about 15% of the protein and 20% of the calories worldwide and is a staple food for higher than 200 million population (Chomba et al., 2015). Corn is the main cause of micronutrients and phytochemicals including anthocyanins, carotenoids, and phenols, which is critical for disease prevention (Goredema-Matongera et al., 2021). In addition to its adoption as human nutritional food, corn commits substantially to the cycle of livestock-to-meat worldwide and serves many industrial directions, including the production of liquor and biofuels (Boddupalli et al., 2020). Human health problems are associated with micronutrient deficiencies worldwide, particularly in developing countries, but the productivity of livestock raised in these countries is also negatively impacted. The low levels of Phyto available micronutrients in cultivated soils and commonly consumed food and forage crops are the main apprehensions for the high prevalence of micronutrients lacking in humans.

Micronutrient deficiencies in soils are not unusual to place in each evolved and developing nation. Soils of Western Balkan nations fluctuate significantly with the concentration and availability of micronutrients, inclusive of Zn, Fe, and Se, as their availability is affected via way of means of soil factors, inclusive pH, soil natural matter, fertilization application, micronutrient concentration (Grujic et al., 2021). Deficiencies of zinc and Iron are rated fifth and sixth, correspondingly, among the dominant factors of risk responses to the global anxiety of disease. These deficiencies are most familiar in children and highly familiar in ladies because of the shortage of blood during the menstrual cycle and childbirth. In addition, micronutrient deficiencies in developing countries are compounded by poverty of knowledge and managing of diversified and equitable foods and dietary manners and an immense prevalence of infectious diseases. To address nutrient deficiencies, minerals, in particular, different interventions have been highlighted including B. Food diversity, implementation of pharmaceutical approaches, and fortification technology (Khayum et al., 2019).

1.1. Malnutrition in maize crop:

A huge strength of people around the world deteriorates from “hidden hunger or micronutrient deficiencies”. The diet of indigent people in dynamic countries generally subsist of a large aggregate of staple cuisines, but a minority of the enriched micronutrient foods such as fresh fruits, vegetables, and animal and fish nutritional products. People across Asia depend on corn, but the staple food falls short of daily nutritional needs and is lacking in fundamental vitamins including vitamin A and specified nutrients including iron and zinc (Murdia et al., 2016). Corn has a high level of carbohydrates but is limited for other micronutrients i.e: imperative amino

acids such as lysine and tryptophan. Corn endosperm is poor in essential minerals such as Iron and Zinc. Due to the over-dependence on corn in Asia, nutrient-associated disorders including Kwashiorkor and pellagra which are acquired by protein and deficiency of tryptophan, correspondingly, night blindness caused by the deficiency of vitamin A, and intense respiratory infections activated by the deficiency of zinc (Dube et al., 2018). Unfortunately, although corn kernels provide several macro-and micronutrients for human metabolic synthesis needs, levels of various essential nutrients, including zinc, are insufficient for consumers who rely on corn as their staple diet.

Zinc is crucial for the advancement and health of young children (Chomba et al., 2015). For the health of living organisms, zinc is a very important element on earth (Potarzycki and Grzebisz, 2009). Zinc exclusively takes on a huge number of objective keys; because it is mandatory for good human health, it is also referred to as the "metal of life". Zinc behaves like an antidepressant to overcome depression. Patients with discomfort from a couple of genetic disorders, including Alzheimer's and Parkinson's disease have lower blood zinc levels. Zinc plays a key role in regulating the blood pressure of humans. In arterial hypertension, zinc levels are reduced in lymphocytes, blood serum, and bones, while the level in erythrocytes increases in the heart, liver, kidneys, adrenal glands, and spleen. Zinc plays a key role in inhibiting hepatitis and liver cirrhosis. Zinc also administers the operations of the endocrine system by modulating the activity of the thymus gland (Maqbool and Beshir, 2019). Most of the engaged and composed corn is white and does not contain a large number of micronutrients. This may partially elaborate on why vitamin A deficiency (VAD) is the dominant problem of public health in the world. This deficiency affects roughly 33 million children in preschool in Africa (Pillay et al., 2014) and is answerable for almost 20-24% of infant fatality from the common diseases of diarrhea, measles, and malaria and 3% of fatality from a large number of infectious diseases in the country of South Africa, the number of children with VAD has expansion from 33% in the year of 1994 to 64% in the year of 2005(Pillay et al., 2014). Struggles to mitigate VAD have included the arrangement of dietary minerals, fortification of refined foods, advancement of diet diversity, and more new biofortification of predominant foods. Biofortification improves or modifies predominant foods to have higher absorption of provitamin A carotenoids or other several micronutrients (Stevens and Winter-Nelson, 2008).

1.2. Biofortification of Maize:

Biofortification is the handling technology of crop improvement manners and advanced biotechnology approach to producing predominant crops rich in micronutrients. The biofortification approach plan targets the indigent living in remote agrarian areas, which are among the hardest to reach vitamin A supplementation and fortification programs (Li et al., 2010). The term biofortification is generally attributed to the biological enhancement of food crops with the majority of micronutrients through agronomic methodologies, conventional plant breeding approach, or genetic engineering of the food crop. Several enriched corn varieties have

been commercialized over conventional and molecular-based breeding approaches, but enriched corn has only been developed for definitive attached nutrients, be it Zn, provitamin A, lysine, and tryptophan (Goredema-Matongera et al., 2021). Typical advantages of biofortification are that it ensures a regular daily supply of nutrients in all age groups and is self-sufficient and profitable after implementation in agricultural systems (Dube et al., 2018).

The successful application of the biofortified approach is: (1) growing a high-yielding worthwhile biofortified variety (2) demonstrating its effectiveness in lowering malnutrition and (3) making sure that biofortified crop is suitable for farmers and consumers. further, any other important requirement is the advent of attention and demand for the biofortified crop and its food (Pixley et al., 2013)

The current competitor of zinc deficiency is mainly associated identical with diet systems occupied by grains such as wheat, rice, and corn. Recognizing the outstanding concern of Zn for human health, the recent analysis focuses on the improvement of Zn-biofortified maize crops using various approaches to combat malnutrition and also discusses the challenges related to the circulation of Zn-enriched maize crop genotypes. Maize was chosen on the point of target byproduct because it is one of the dominant food crops in Zn-deficient regions.

2. Implementation of Biofortification

There is a series of activities for the scientists to increase the nutritional status of a maize crop which is classified into three stages discovery, Expansion, and Distribution. These stages will illustrate the impact of nutritional status by identifying, validating, and discovering the nutrient target (Bouis et al., 2011). The successful biofortification can be achieved by approaching the following impact pathway shown in figure 01.

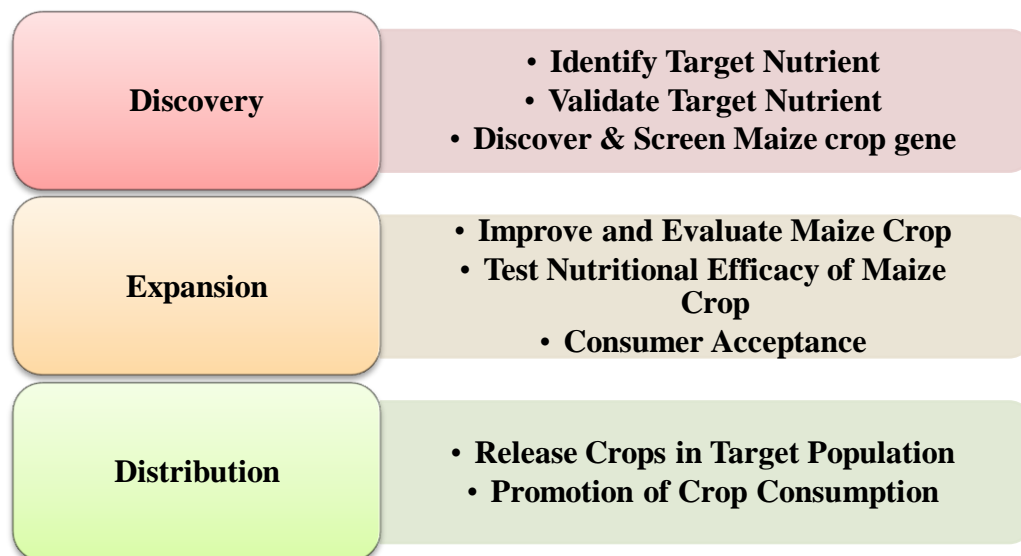


Figure 01. Strategy for the Improvement of Maize Crop Nutrition

3. Advance position of biofortified maize crop:

HarvestPlus has started an international integrative affiliation of scientific research associations and implementing departments in the biofortification strategy. The “Bill and Melinda Gates Foundation-funded Grand Challenges 9” is crumbling many transgenic crops. Success made with maize crop is summarized in table 01 and the micronutrient content level of maize crop, across varieties from HarvestPlus screening movements, are shown in figure 02.

Crop Name	Essential Nutrient	Targeted Country	Pointed Institutions	First Release Year
Maize	Zinc	Africa	Wageningen University	2016
	Provitamin A	Zambia	CIMMYT,IITA,ZARI	2012
	Carotenoids	Nigeria	CIMMYT,IITA,IAR & T	2012
		Brazil	Embrapa	2013
		China	Institute of Crop Science, YAAS	2015

Table 01. Biofortified maize crop and its countries release schedule. CIMMYT, International Maize and Wheat Improvement Center; IITA, International Institute of Tropical Agriculture; IAR&T, Institute of Agricultural Research and Training; ZARI, Zambia Agriculture Research Institute; YAAS, Yunnan Academy of Agricultural Sciences.

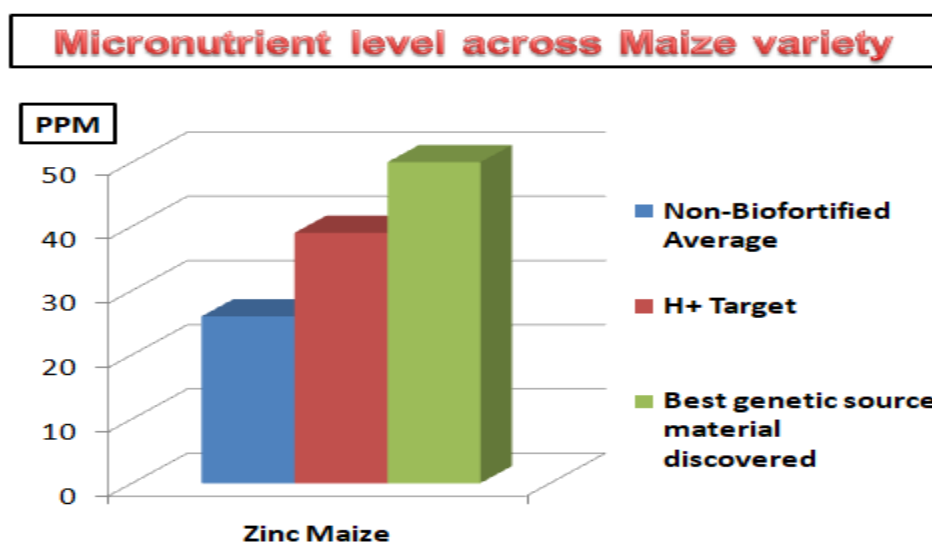


Figure 02: The micronutrient content level of maize crop, across varieties from HarvestPlus screening works.

4. Approaches used to make biofortified maize:

Most of the world relies on grain-based staple foods. Grain-based staple foods, which are generally poor in micronutrients, particularly Zn and Fe, more than 2 billion people are affected by an insidious sort of deficiency called micronutrient malnutrition. Even small amounts of micronutrient malnutrition can impair reduce disease resistance cognitive development, and raise the risk of death in women throughout childbirth. The micronutrient fertilization application has been shown to increase the yield and nutrient level of forage crops. Agronomic biofortification offers a direct or good method to improve the agglomeration of micronutrients, mainly Fe and Zn, in cereals (Jan et al., 2020).

4.1.Maize agronomic biofortification:

Agronomic biofortification is a simple or inexpensive application to improve the nutrient level of food. Three techniques of Zn through-soil fertilization, foliar spraying, or seed dressing are especially favorable in Zn lacking soils to allow the herb to take up the micronutrient straightly, involve in a remarkable improvement in crop yield and growth. Extractable Zn-enriched loam increased the concentration of Zn in maize tissue and grain by 6-fold and 37%, respectively.

Improving soil Zn fertility permits plants to consume further Zn and distribute it to grains and other plant parts. Zinc oxide, zinc chelates, and Zinc sulfate are the most common mineral Zn fertilizers in the soil. Soil fertilization with Zn ($50 \text{ ZnSO}_4 \cdot 7\text{H}_2\text{O} \text{ kg ha}^{-1}$) increased the maize grain yield significantly. A 3-year of field analysis of corn shows that 11 kg of Zn per hectare is needed to expand Zn availability for optimal corn building (Obaid et al., 2022).

4.1.1.Foliar fertilization for the biofortification of maize:

Foliar fertilization is described as the foliar spraying and implementation of one and more vital plant mineral nutrients to aerial parts of plants for traditional soil fertilization. Many nutrient fertilizers are water-soluble and can be applied directly to the above-ground parts of the plant. Abandoned nutrients can invade the leaves through numerous stairways, pervasive the cuticle and entering between stomata, prior entering to the plant cell in which they can be pre-owned in metabolism (Alshaal and El-Ramady, 2017). Foliar spraying is also useful to combat Zn shortfall in standing crops batch for example ZnO nanoparticulate concentrated sprayed in the milking and ear phase, has an outstanding effect on brain growth(Martínez Cuesta et al., 2022).

The concurrent administration of organic fertilizer alongside Zn fertilizer in maize-legume mixed culture methods can enhance the Zn concentration within the maize kernel (Botoman et al., 2020). The advantage of the combination of organic or mineral fertilizers in the form of Zn, NPK, or litter lies in the provision of P or Zn residues for the following maize harvest. The mixture of multiple Zn fertilization applications (spreading + leaf or banding + leaf) is more successful than a sole approach to improve the concentration of Zn in grain and further parts of maize (Obaid et al., 2022).

4.1.2. Microbially-mediated biofortification of maize:

Microbially-mediated Zn biofortification is one of the most accessible, simple, and authentic strategies to enhance the accumulation and concentration of Zn in the digestible part of the maize plant. Our results demonstrated that residual Zn fertilization is a viable and sustainable technique that increased plant growth, yield, and Zn nutrition in both growing seasons. Inoculation of diazotrophic bacteria together with residual Zn fertilization showed better performance than no Zn fertilization treatments. Inoculation of the seed of *A. brasilense* and *B. subtilis* has the plug height of the first productive spike, plant height, shoot dry matter, and grain yield of maize under residual Zn fertilization (Ghosh et al., 2017)

4.1.3. Maize seed priming:

Priming of seed is the analysis of seeds with different nutrients and solutions before sowing. Frequently worn practices are osmopriming and hydro priming. Priming of seed with ZnSO₄ showed a 27% increase in corn yield. Various studies have delineated the increase in grain yield, stubble yield, and biotic yield in maize by ZnSO₄ priming of seed. Preparing the seeds alongside Zn solutions boosts the content of Zn corn kernels at maturity with other characters. It was also found that the mixed application of seed primer with Zn foliar application resulted in a 43.61% increase in maize grain hybrids (Maqbool and Beshir, 2019).

4.2. Conventional breeding techniques:

Common breeding approaches, any selection to improve hybridization and population (heterosis breeding), are functional to improve grain concentration of Zn. The International Maize and Wheat Improvement Center undergo over forty years of professional knowledge with full dedication to the development of a corn. About 50 QPM diversity of high-quality protein corn should be introduced in Latin America. QPMFourteen hybrids are launched, 2 in Pakistan and one in India. CGIAR MAIZE program and HarvestPlus are collaborating with the CIMMYT to develop or implement biofortification of maize. Recently, more than forty provitamin A-enriched genotypes of maize, together with hybrids and open-pollinated varieties, have been commercialized in numerous countries of Africa. In Guatemala, nearly 46.5% of children below the age of 5 suffer from dwarf growth and 29.8% of the population is dietary deficient in Zn, while in Colombia nearly 22% of the population is deficient in Zn. There are few domains such as the Amazon and pacific coast in which up to 65% of the population is Zn deficient. In Guatemala, the Zn-enriched corn varieties ICTA HB18 and ICTAB15 were developed. Guatemala is the first country to commercialize Zn-enriched corn hybrids. ICTA HB18 has 15% more Zn than other profit-oriented grades, while tortillas built with ICTA B15 contain almost 60% more Zn than tortillas made with more commercial grades. BIOMZN01 is a Zn bioenriched corn variety started by CIMMYT. BIOMZN01 has 36% more Zn in the grain than other varieties, which means that arepas, one of the Colombian dishes, can contain all of 5 times more Zn when prepared with this new variety. BIO-MZN01 has a yield potential of up to 8 tons per

hectare (t/h) and is tolerant to regional corn diseases such as Turcicum blight, rust, and gray spot. It can also produce in a variety of latitudes from 0 to 1400 m throughout both seasons of growing in the country. Not only is BIOMZN01 enriched in Zn, but it also has many other properties that make it high-yielding and disease-resistant (Maqbool and Beshir, 2019).

4.3. Transgenic approach for developing biofortified maize:

The genetic engineering of corn to grow nutritionally corn genotypes of upper level could be a good biotechnological breeding approach that could reduce some of the barriers breeders face in developing multi-nutrient corn. Many techniques have been offered to incorporate transgenes into the genome of maize, including microparticle bombardment, whisker-mediated transformation, and *Agrobacterium tumefaciens*-mediated transformation. Various studies have accounted for the successful development of varieties of transgenic biofortified maize. For example, successful integration of the sb401 transgene encoding a lysine-rich protein into the maize genome was found to enhance lysine level and total protein levels in the transgenic amount of QPM. A familiar transgenic strategy was used to enhance natural Zn- and QPM-enhanced white or yellow genotypes high in provitamin A by giving overexpression of the bacterial genes such as crtB and crtL, leads to a 34-fold increase in the development of total carotenoids in maize crop endosperm (Goredema-Matongera et al., 2021).

The genetic biofortification technique improves protein, provitamin A, and Zn content in corn crop. The studies demonstrated positive factors of bioefficacy from the utilization of enriched corn porridge, ie, β -carotene to retinol, in US ladies, even though in short-term experimental settings. The child of Zambian between the ages of 5 and 7 years demonstrated a powerful expansion in total body stores of the nutritional level of vitamin A after 3 months of the utilization of biofortified maize with provitamin A. Meanwhile, rural Zambian children under the age of 5 reportedly benefited from consuming biofortified maize crops with a high intake of Zinc components in their bodies (Obaid et al., 2022).

4.3.1. CRISPR-mediated biofortification:

The editing of a genome is a powerful bioengineering strategy that can be used to store nutrients in corn. Approaches of zinc finger nucleases (ZFNs) and transcriptional activator-like effector nucleases (TALENs) have been extensively used for genome editing purposes. A recent development in genome editing techniques such as “B. Clustered Regulatory Interspaced Palindromic Repeats” (CRISPR) empowers accurate modifications of the genome with more reproducibility and avoids cell toxicity. For example, yield and stress tolerance in rice β -carotene content in Cavendish banana reduced phytic acid content in corn kernel, and powdery mildew resistance in wheat and drought-tolerant corn. Therefore, multi-nutrient maize can be developed using state-of-the-art technologies that have great potential to gain wider public acceptance compared to GM crops.

4.3.2. Marker-approved breeding:

Marker-approved or assisted breeding could enhance the development in the development of multi-nutrient maize. Marker-approved or assisted breeding technique is an ambiguous selection process that involves selection for the devoted trait based on a specific marker, which can be morphological, biochemical, or DNA/RNA markers (known as molecular markers). MAS can also be used to confirm the presence of the opaque2 locus in QPM donors used for QPM-based multi-nutrient corn. (Goredema-Matongera et al., 2021).

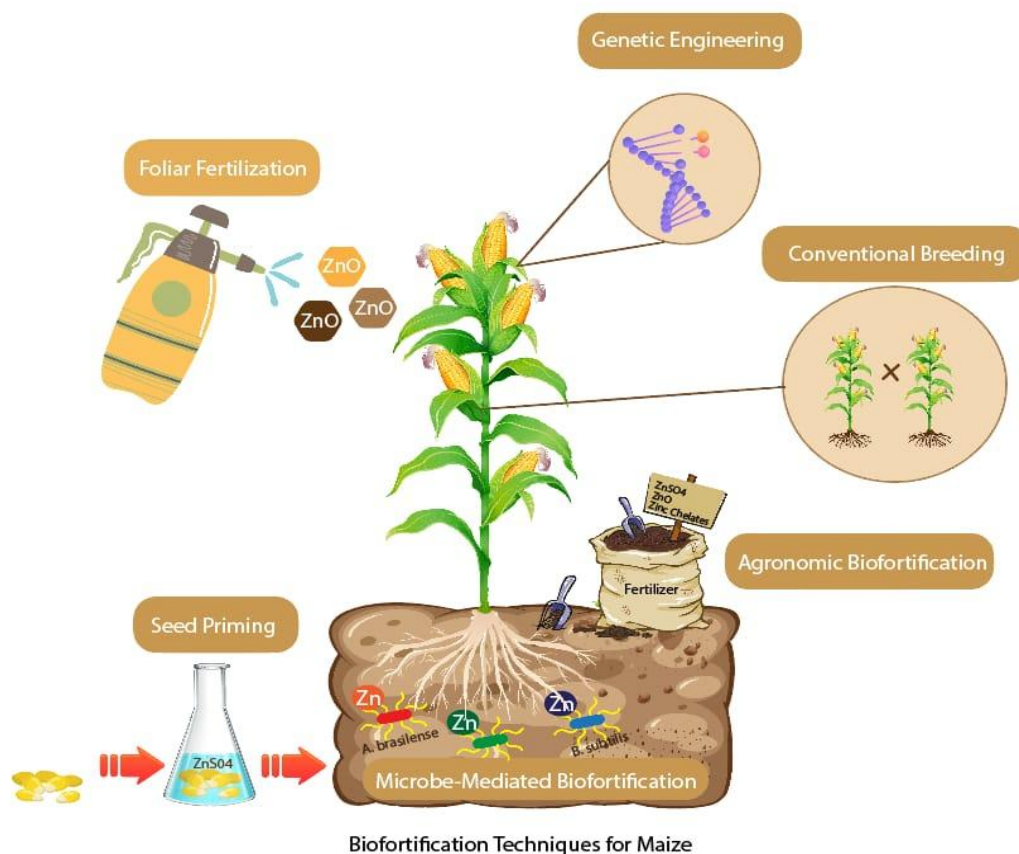


Figure 03. Illustration of biofortification Strategies; agronomic approaches, conventional breeding, genetic engineering, and agronomic biofortification in maize to improve micronutrient content. All of the approaches are useful to increase the concentration of Zn in grain.

5. Proficiency of Biofortification:

The fundamental indication for the efficacy of biofortification arrives from an orange sweet potato. The influence of biofortification was retrieved through an irregular control trial in the countries of Uganda and Mozambique. This project resulted in a 68% enhancement in the probability of orange sweet potato maintenance in Mozambique and a 61% enhancement in

Uganda country (Hotz et al., 2012). Provitamin A maize breeding is administered by the International Maize and Wheat Improvement Center (CIMMYT) and International Institute of Tropical Agriculture (IITA) with the partnership of NARES in the country of Southern Africa. The screening of germplasm identified genetic changes for the matched target (15ppm) of Provitamin A micronutrient in the maize, which was bred into equatorial varieties. The current revolutions in the technology of marker-assisted selection have boosted the efficiency and speed of analyzing genes that regulate the targeted trait of the maize crop. In 2012, varieties were released that can contribute 25% of the EAR for young women and children of preschool in Nigeria (2 varieties) and Zambia (3 varieties). In the USA, Embrapa of the Brazil country has certified the variety of maize crops for release that has identical levels of Micronutrient, Provitamin A. A number of varieties are in the developmental criteria that can afford 70% of the EAR. Nutrition of vitamin A losses between 25% in the food and cooking processing methods (Li et al., 2007). The bioavailability of Provitamin A as with maize crop is much superior to pretended; the beta carotene conversion into retinol has been deliberated with and without enumerated oil at 3.8 to 01 and 4.3 to 01, subsequently (Li et al., 2007, La Frano et al., 2013).

6. Assurance and potential Hurdles for biofortified maize crop

6.1. Consumer acknowledgment and farmer acceptance:

Agrarian Consumers always wish for high nutritious food and desire to pay a premium amount for it, arguing favorable assessment of and demand for maize crop full of nutritional perks. The previous studies on “willingness-to-pay” for orange maize illustrate that the consumers admired the sensory attributes of the biofortified maize crops and will allowance a high amount of high level of Provitamin A varieties in comparison to white varieties (Meenakshi et al., 2012).

6.2. Maize Crop Performance

The high quantity of minerals and vitamins can be bred into the succulent sections of maize crops, meantime controlling high nutritional values, defending against pests and crop diseases, and more fascinating agronomic traits. Without these traits, the farmers will reject utilizing biofortified maize crops; every variety discharged must be partially aggressive with what is accessible to the market.

6.3. Transgenic biofortified maize crop

Currently, many transgenic crops are under development that also have traits of agronomic behaviors i.e. disease resistance.

Meanwhile, transgenic methodologies may decrease time-to-market; protection to obtaining transgenic maize crops lasts high, especially in the country of Africa

Conclusions:

To overcome the major disagreements concerning the knowledge of biofortification, higher efficacy of research trials and effective approaches are needed to establish and augment the encouraging data thus far collected. Scientific researchers should further clarify indicators of respective micronutrient levels and improve the understanding of the importance of cross nutrient synergism. Marketing Research will enhance the efficacy of additional delivery and marketing research approaches in providing the maximum level of consumption and adoption of biofortified maize crop. Breeding can be more expensive by utilizing marker-assisted selection to breed large levels of different micronutrients in a single variety and to accomplish this approach, the transgenic methodology may prove to show more efficacy than the conventional breeding approach. To common traits of biofortification, agricultural research departments must accept conventional breeding for nutrient quantity as an essential activity, contributing to breeding channels at the National Varietal release committees (NARES). This committee should be determined to set standards for the better quality of nutrients in the maize crop that is discharged, presently only agronomic approaches are recognized.

The agricultural food systems are very essential in providing most of the compounds and nutrients that humans feel are a necessity for the sustainability of health and beneficial lives. In developing countries, agricultural food systems are not working well due to environmental constraints human capacities, high population levels, and institutional restraints. So, biofortification is an advanced technology, playing important role in the enhancement of nutritional crops and ensuring a more nutrimental future, but the investment in this technology may seem cost-effective.

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