

Review

Advances in Genetic Enhancement of Nutritional Quality of Tropical Maize in West and Central Africa

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Abstract: Micronutrient deficiencies are pervasive in the diets of millions of people in developing countries, calling for effective mitigation measures. The development of biofortified cultivars through breeding holds promise for sustainable and affordable solutions to combat micronutrient deficiencies. Breeding efforts in the past decade have resulted in dozens of biofortified open-pollinated varieties and hybrids adapted to diverse agroecological zones. Advances in genomics and molecular tools enabled rapid identification of maize cultivars enriched with essential micronutrients such as pro vitamin A (PVA), iron (Fe), and zinc (Zn). Leveraging Multi-omics-driven discovery of the genetic factors underlying the vast array of nutritional traits is paramount to mainstreaming breeding for quality traits in the product profile. Molecular breeding schemes, and integrating emerging Omics tools at every stage of the breeding pipeline, are vital to enhancing genetic gain. The recent momentum in elucidating the metabolism of micronutrients should be expanded to novel breeding targets as well as to the simultaneous enhancement of nutritional qualities while curtailing anti-nutritional factors in staple food crops. Harnessing new technologies to establish comprehensive and integrated breeding approaches involving nutrigenomics, genome editing, and agronomic biofortification is crucial in tackling nutritional insecurity. This review highlights the prospect of integrating modern tools in hastening the genetic improvement of nutritionally enriched maize.

Keywords: West and Central Africa; biofortified maize; micronutrient; breeding; nutritional quality



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1. Introduction

The history of maize breeding at the International Institute of Tropical Agriculture (IITA) dates back to the 1970s, with the main focus on productivity and biotic stress tolerance [1]. One of the earliest endeavors in improving the nutritional quality of IITA was the development of maize germplasm with enhanced levels of lysine and tryptophan (also known as quality protein maize (QPM)). Studies on micronutrient accumulation had not been initiated until early to mid 2000s, when breeding biofortified maize in Africa began as a concerted effort of research institutions and organizations, with the aim of addressing nutritional challenges and improving the well-being of communities across the continent [2,3]. The prominent players in this concerted effort to combat micronutrient deficiencies are the International Maize and Wheat Improvement Center (CIMMYT) and IITA, and the Zambia Agriculture Research Institute [4] began in 2007 and have operated at full scale since 2011, alongside others [5].

It has been demonstrated that genes and genomic research are drivers of accelerated and efficient improvement in productivity, resilience to stresses, and enhanced nutrition in maize. Hundreds of genetically enhanced provitamin A and mineral-rich cultivars are cultivated by millions of small-scale farming communities in developing countries [6,7]. Africa is the most affected region of the world, with a large number of undernourished people. Notably, micronutrient deficiencies are crucial problems in the diets of millions of people in developing countries, as depicted using various indicators of malnutrition (Figure 1), calling for effective mitigation measures. Vitamin A deficiency, in particular, affects millions of

subsistence farming families in Africa, inflicting numerous preventable infectious diseases. The challenge, therefore, is to deliver nutritious, safe, and affordable food to reduce the impact of nutritional insecurity. Several interventions have been attempted worldwide in order to overcome the chronic problem posed by micronutrient deficiencies. However, most of these approaches were not entirely successful due to ineffective distribution systems [7], insufficient infrastructure, and/or non-affordability [8].

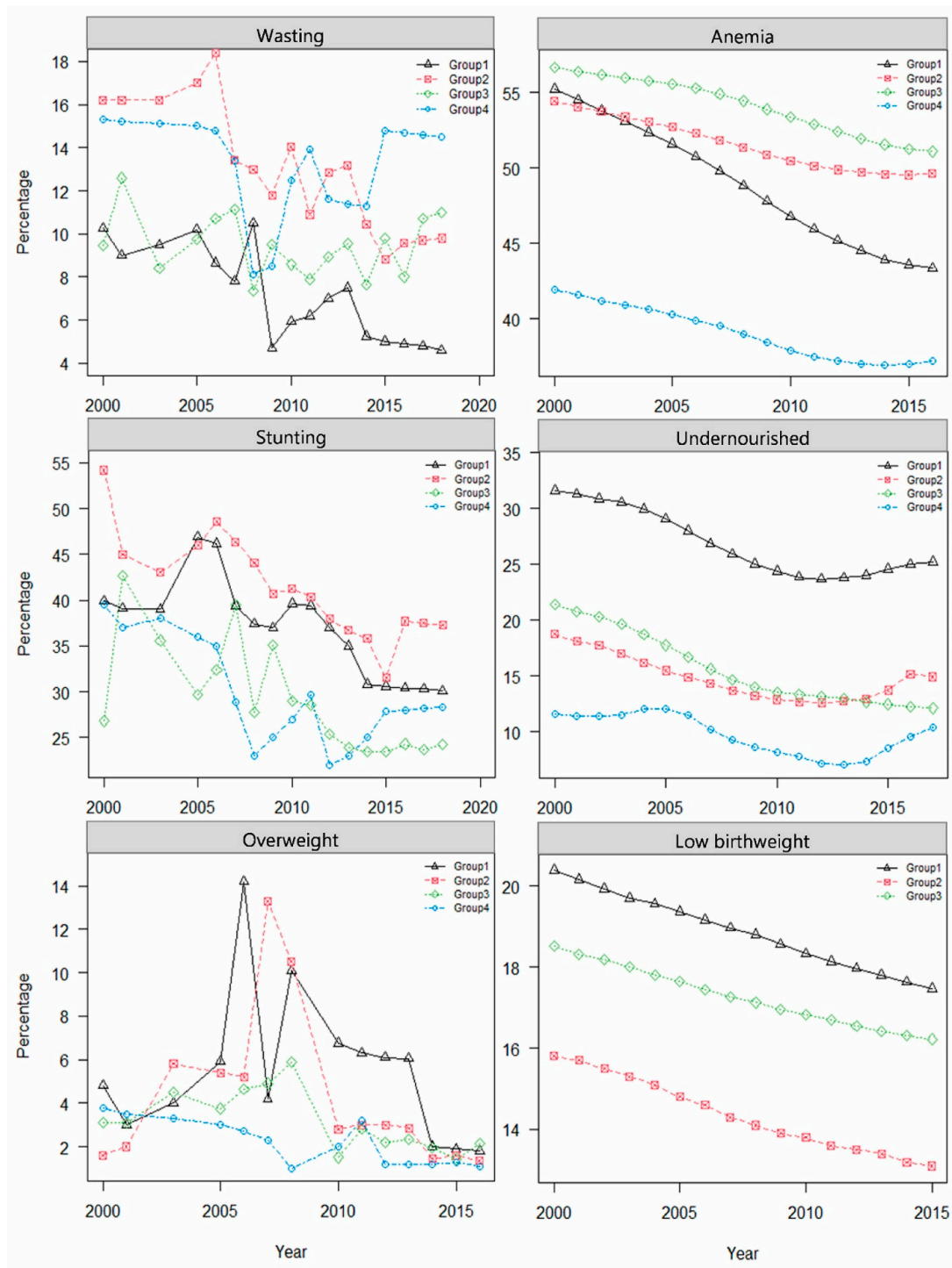


Figure 1. Evolutionary trends in six malnutrition/hidden hunger indicators over time (1999 to 2018) regarding the realized clusters of countries in West Africa (G1: Liberia, Benin, Sierra Leone, Guinea Bissau. G2: Niger, Burkina, Nigeria. G3: Côte d'Ivoire, Guinea, Ghana, Togo, Mali, Gambia, Senegal. G4: Mauritania). Adapted from Chadare et al. [9].

The development of micronutrient-enriched staple plant foods through breeding holds promise for sustainable and affordable food-based solutions, to combat micronutrient deficiencies with a broad spectrum of health benefits, in preventing the rampant diseases ravaging people with low incomes in developing countries. Nutrient-enriched crops would also serve as a logical vehicle for providing micronutrients in pure form in diets [2,10]. Micronutrient deficiencies are pervasive in developing countries' rural populations, where they mainly rely on cereal-based diets as a staple food. Yellow maize is an essential dietary source in various West African countries and is consumed in different forms [11]. Therefore, boosting the nutrient content of maize kernels through genetic improvement is a reasonable means to lessen the adverse effects of micronutrient deficiency. The benefit of yellow maize could be attributed to both provitamin A and non-provitamin A carotenoids and other essential micronutrients that have different and complementary roles in maintaining human health [12]. Zinc (Zn) and iron (Fe) are essential minerals and nutrients important in various biochemical pathways and biological processes. As co-factors that catalyze about 300 enzymatic reactions, they are involved in the metabolism of macromolecules, such as proteins, carbohydrates, and lipids, including controlling gene transcription [13,14]. The genetic framework underlying the uptake, mobility, and accumulation of Zn and Fe is a complex process regulated by many genes [14]. Several studies have been conducted to identify and characterize QTLs associated with micronutrient concentrations in maize [13–15]. The results from these studies corroborate the identification of genomic regions associated with Zn and Fe contents in maize with potential crop improvement and nutritional enhancement through transgenic biofortification or marker-assisted selection.

The accelerated development and deployment of biofortified maize in several African countries is the outcome of a modern breeding scheme that has benefited tremendously from advances in genomics and molecular tools, which enabled rapid identification of cultivars that contain recommended PVA levels and still maintain productivity and other desirable agronomic and adaptive traits [7,12]. In general, biofortified maize is getting wide acceptance in Africa and beyond. In sub-Saharan Africa (SSA) alone, 69 provitamin A-enhanced varieties were conventionally developed and released to farmers. These varieties are competitive in terms of grain yield and other economically important traits in addition to their nutritional quality as compared to the typical varieties being produced and consumed by farmers in SSA. This review will highlight the effort to integrate modern tools in hastening the genetic improvement of nutritionally enriched maize hybrids and open-pollinated varieties (OPVs).

2. Breeding for Enhanced Nutritional Quality

Earlier studies in the International Institute of Tropical Agriculture (IITA) revealed the presence of genetic variations in carotenoid content in tropical maize inbred lines, but the levels of PVA carotenoids were low, prompting the introduction of exotic germplasm to enhance carotenoid content [16]. At IITA, breeding for nutritionally enriched tropical maize varieties and hybrids was performed in collaboration with other stake holders in both the public and private sectors. Notably, the global HarvestPlus Challenge Program (<https://www.harvestplus.org/> (accessed on 6 February 2024)) made significant investments in IITA and partners to enrich maize with provitamin A through conventional breeding, which resulted in the development and release of nutritionally enhanced maize hybrids, open pollinated varieties, and elite parental inbred lines. Furthermore, IITA collaborated with CIMMYT (cimmyt.org) and other research institutions to exchange germplasm and molecular tools vital for nutritional enhancement of maize. The IITA maize breeding program involves collaborative testing of nutritionally enhanced maize varieties in different countries in the region. In addition to the public research organization, private seed companies in the region play a crucial role in the testing and release of nutritionally enhanced maize varieties. Over the years, biofortified maize varieties have been disseminated to farmers across various African countries through seed distribution programs, research collaborations, and partnerships with local agricultural institutions. These efforts have

contributed to increasing the availability of nutrient-rich maize varieties and promoting their cultivation among smallholder farmers in Africa. This approach harnessed novel genes controlling high levels of β -carotene and other carotenoids ($>15 \mu\text{g g}^{-1}$) from temperate and tropical maize germplasm to boost provitamin A in tropical-adapted maize. This endeavor led to the development of many varieties and hybrids with high provitamin A content ($>15 \mu\text{g g}^{-1}$) without compromising grain yield and other adaptive traits that are required to cultivate maize profitably by farmers in Africa [17]. The involvement of farmers as strategic partners in conducting site-specific on-farm trials, where their preferred maize varieties and hybrids have been compared side by side with promising provitamin A-rich varieties, has facilitated the selection of the best maize varieties for registration, release, and commercialization. As a result, 18 varieties and 22 hybrids with intermediate to high levels of provitamin A were released in Cameroon, the Democratic Republic of Congo, Burundi, Ethiopia, Ghana, Mali, Nigeria, and Zambia from 2012 to 2022. In West Africa, over 34 open-pollinated varieties (OPVs) and hybrids developed by IITA and national program partners have been released (Table 1). Some of the released maize varieties and hybrids have provitamin A content that meets the breeding target of $15 \mu\text{g g}^{-1}$ set by the HarvestPlus Challenge Program. So far, 76 vit A fortified maize OPVs and hybrids, adapted to diverse agroecological zones, were released in 11 African countries between 2012 and 2023 (https://bcr.harvestplus.org/varieties_released_list?crop=Vitamin_A_Maize (accessed on 6 February 2024)).

Table 1. Lists of pro-vitamin A-rich maize varieties developed by IITA and partners and released in the West African countries of Ghana, Mali, and Nigeria.

IITA Name	Variety Name	Year of Release	Country	Yieldt/ha	PVA ($\mu\text{g/g}$)	Yd Adv (%)	Cultivar Type
A0905-32	Ife-Hybrid 4	2012	Nigeria	5–8	7–11	16	SC hybrid
A0905-28	Ife-hybrid 3	2012	Nigeria	6–8	7–10	27	SC hybrid
PVASYN 2	Sammaz 38	2012	Nigeria	4–6	8–9	47	OPV
PVASYN 8	Sammaz 39	2012	Nigeria	4–6	8–10	48	OPV
PVASYN 6	CSIR-CRI Honampa	2012	Ghana	4–6	8–9	42	OPV
LY1001-21	Sammaz 43	2015	Nigeria	6–9	7–10	42	TW hybrid
LY1001-14	Sammaz 44	2015	Nigeria	6–9	7–10	59	TW hybrid
LY1001-10	Dzifoo	2015	Ghana	6–9	7–10	45	TW hybrid
LY1001-14	Ahoofe	2015	Ghana	6–9	7–10	45	TW hybrid
PVA SYN-13	Ahoodzin	2015	Ghana	4–6	8–12	29	OPV
PVASYN 7	MUIBAKI 1	2016	DRC	4	5–6	14	OPV
PVASYN 9	MUIBAKI 2	2016	DRC	5	6–9	25	OPV
PVASYN 13	MUIBAKI 3	2016	DRC	5.5	8–12	35	OPV
PVASYN 3	Nafama	2012	Mali	5–6	6–8	0	OPV
PVASYN 13	Kodialan	2016	Mali	5–7	8–12	0	OPV
PVASYN 21	Dakan	2016	Mali	7–8	6–7	40–60	OPV
LY1001-18	Abebe	2016	Mali	9–10	8–13	80–100	TW hybrid
LY0905-32	Duba	2016	Mali	8–9	7–10	60–80	TW hybrid
LY1001-10	Sammaz 49	2016	Nigeria	5–8	6–11	10–29	TW hybrid
PVASYN 13	Sammaz 52	2017	Nigeria	5–6	8–12	31–57	OPV
LY1501-5	CRI-Nkwagye	2018	Ghana	8–9	10–14	36	TW hybrid

Table 1. Cont.

IITA Name	Variety Name	Year of Release	Country	Yieldt/ha	PVA (µg/g)	Yd Adv (%)	Cultivar Type
LY1501-9	CRI-Abebe	2018	Ghana	8–9	11–15	47	TW hybrid
PVA SYN 6	NA	2018	Cameroon	4–6	8–9	10	OPV
PVASYN 13	NA	2018	Cameroon	5–6	8–12	10	OPV
LY1501-8	SAMMAZ 57	2019	Nigeria	6–8	11–15	62–89	TW hybrid
PVA HGAF2	BATA	2019	Mali	8–9	7–10	60–80	OPV
F2SCA1413-36	SAMMAZ 59	2020	Nigeria	4–8	13–16	18	OPV
F2SCA1413-12	SAMMAZ 60	2020	Nigeria	4–7	13–16	27	OPV
LY1001-23	ZAMS666A	2021	Ghana	5–8	8–10	33	TW hybrid
LY1409-21	CRI-Ewool	2021	Ghana	4–7	9–14	12	TW hybrid
LY1501-7	CRI-Harvestplus	2021	Ghana	5–7	10–13	9	TW hybrid
A1802-4	SAMMAZ 67	2022	Nigeria	5–7	10–13	19	OPV
LY1606	HAKIMI 1	2022	Nigeria	5–7	11–12	11	OPV
LY1608	SAMLAKE 1608MY	2022	Nigeria	5–7	12–13	20	OPV

Yield advantage over farmer's preferred variety/Hybrid check expressed in %; PVA, Pro-vitamin A content (PPM); SC hybrid, single cross hybrid; TW hybrid, three way hybrid.

Between 2014 and 2021 alone, a total of 27,337 tons of certified seeds of provitamin A biofortified maize varieties were produced in Ghana, Mali, and Nigeria and sold to farmers that planted an estimated 1,093,464 ha, benefiting 2,733,661 households in the rural areas and urban centers, estimated based on an average household size of 10.3 in these countries. In a national food consumption and nutrition survey conducted in Nigeria in 2021 and 2022, 13.4% of the respondents consumed provitamin A biofortified orange maize or foods produced from it in the past 30 days of the interview. The consumption of provitamin A biofortified maize was 38% in the Northeast of the country due to support programs from the Federal Government of Nigeria and donor agencies in response to the insurgency in this zone [18]. Provitamin A biofortified maize has also increased competitiveness through increased use in various food products and targeting niche markets in the country. The number of biofortified maize varieties in Africa exceeds that of other crops (Accessed from HarvestPlus website in May 2023, <https://www.harvestplus.org/home/global-reach/> (accessed on 6 February 2024)).

2.1. Simultaneous Improvement of Nutritional Quality Traits

The prevailing nutritional facts in SSA reveal the imperative to increase the level of several micronutrients concurrently. The product profile of an acceptable candidate variety, that was nominated for release, comprises adaptability and stress resilience, nutritional quality, and high productivity. Breeding for so many different traits, however, is cumbersome and entails different strategies depending on the genetics of the trait. Fortunately, many nutritional quality traits are considered less complex and highly heritable, paving the way for simultaneous improvement of the desired priority attributes. Furthermore, the biochemical and molecular basis of carotenoid biosynthesis has been thoroughly described over decades [6,19]. The latter prompted the development of molecular tools to aid genomics-assisted breeding for several quality traits, mainly PVA and quality protein maize (QPM). While the work on essential minerals, such as Fe and Zn, is in progress, more effort should be made to accelerate the development of trait-linked markers. Meanwhile, it is imperative to design a breeding scheme that aims to pyramid several quality traits by using hitherto developed tools [6,7,20]. Candidate varieties with multiple traits must

be tested in numerous locations across target environments and production systems to validate genotype \times environment (G \times E) interactions.

Among the mineral nutrients beneficial for human health, iron (Fe) and zinc (Zn) are necessary for growth and development. For the millions of households in low-income countries, VA-enriched maize alone will not adequately reduce the myriad of health issues caused by malnutrition. Since these elements are not synthesized in the human body, crucial micronutrients, such as Fe and Zn, must be delivered through the diet. The genetic improvement of crops to enhance the levels of these elements have been carried out on several food crops, including maize [6,21–23]. The genetic approach of increasing Zn bioavailability or its concentration can be complemented by agronomic biofortification, which includes foliar and soil applications [24]. Due to the impact of diverse edaphic and environmental factors on the genetic expression of the trait, limited progress has been made in breeding tropical maize for high Fe and Zn. Notwithstanding, studies on lowland and mid-altitude maize inbred lines showed genetic variation to consider genetic improvement [25,26]. To date, there are no zinc or iron biofortified maize varieties that have been developed and released in Africa.

The search for early-maturing varieties with high Fe, high Zn, and bioavailable Fe showed significant variations between the elite varieties, of which 34% are attributed to genetics [27]. To assess the existence of sufficient genetic variation in Fe and Zn concentration in tropical maize inbred lines adapted to the lowlands and mid-altitudes, several trials involving different sets of tropical maize inbred lines revealed the presence of significant variation in concentrations of grain minerals among inbred lines in each trial [25]. More recently, multi-environment evaluation of PVA-enriched inbred lines and their hybrids for multiple micronutrients (PVA, Fe, and Zn) identified superior inbred lines and hybrids combining high grain yield with high concentrations of the three micronutrients, demonstrating the possibility of creating nutrient dense maize without affecting grain yield [28].

Presumably, the genetic control of Fe and Zn accumulation is complex. In such cases, molecular breeding, such as GS, is the best genetic improvement approach. Exploring other methods to identify markers for efficient selection could provide additional techniques, such as forward breeding for rapid introgression of these quality traits. The present product profiles can be refined by adding these elements to develop multi-nutrient-enriched tropical maize.

Antinutritional factors, such as phytic acid, reduce the bioavailability of micronutrients owing to their binding affinity for Fe, Zn, and other elements, causing decreased absorption of these minerals. Consequently, breeding for increased Zn was hampered by the negative role of phytic acids [7,12].

2.2. Breeding for PVA and Non-PVA Carotenoids

As the health benefits of the provitamin A (PVA) carotenoids (α -carotene, β -carotene, and β -cryptoxanthin) and the non-PVA carotenoids (i.e., lutein and zeaxanthin) are different, it is worthwhile to select germplasm with a high concentration of both categories instead of selecting for PVA only. A study suggested that lines with favorable alleles of crtRB1 were rich in PVA carotenoids, and those carrying the LcyE favorable alleles were rich in non-PVA carotenoids [29]. However, further investigation is needed to determine the correlation between the two categories of carotenoids and their implication in breeding.

2.3. Breeding for PVA and Protein

Presently, the genetic improvement of maize enriched with PVA is primarily driven by the favorable allele donors' priority. However, sooner or later, the need for other micronutrients will appear in the agenda list. Given the envisaged health benefits of other micronutrients, it is prudent to consider stacking other traits on PVA-enriched maize genotypes using marker-assisted efficient breeding strategies. A recent review article noted the feasibility of stacking multiple traits (QPM, provitamin A, vitamin E, and kernel Zn) using molecular tools without yield penalty [7]. Tackling vitamin A deficiency (VAD)

alone is not sufficient to minimize nutrient-deficiency-caused health problems. Therefore, it is vital to develop a high PVA cultivar enriched with other essential nutrients, such as QPM (lysine and tryptophan) [30]. The availability of markers linked to high lysine and tryptophan in maize stimulated the conversion of PVA lines into QPM and vice versa. Goswami et al. [31] reported a relatively rapid process of converting high QPM inbred lines into high PVA lines using marker-assisted backcrossing (MABC). The process involved using simple sequence repeat (SSR) markers for background selection to recover the recurrent parent genome and foreground selection using crtRB1 linked marker. The advent of SNP markers, amenable to high throughput genotyping, makes such introgression efforts even faster and cheaper.

2.4. Impact of Processing Methods on Degradation and Losses of Micronutrient Content

Maize is consumed/utilized in various ways, impacting the concentration of micronutrients in the final product. The processing methods affect the bio-accessibility and bio-availability of essential nutrients. Breeding for PVA has focused on increasing PVA through the concentration of β -carotene and other carotenoids to the total PVA. However, studies have shown that the PVA available during consumption can be compounded by storage condition, duration, and/or culinary uses and processing. Prompted by the growing popularity of roasted corn in Nigeria, a study revealed that harvesting time impacts the PVA content of roasted corn. Roasting with improvement in husk retained a higher amount of PVA as compared to roasting without husk [32]. Such observation suggests that breeders should target not only the concentration of nutrients in pre-processed kernels but also consider the genetic variation of compounds in post-processed kernels. Based on the various processing methods, new breeding targets spanning processing and culinary traits were suggested [12].

2.5. Association of PVA with Reduced Aflatoxin

Breeding for increased carotenoid content was found to be accompanied by improved aflatoxin resistance in two different studies. Using inbred testers with varying PVA content on aflatoxin-resistant and susceptible lines, Mboup et al. [33] reported a negative correlation between aflatoxin content and PVA accumulation. Likewise, an earlier study involving a cross between aflatoxin-resistant and susceptible inbred lines with varying PVA content revealed that hybrids with higher concentrations of carotenoid have less aflatoxin contamination than hybrids with lower carotenoids concentrations [34]. Understanding the interactions between the genetic factors governing nutritional and antinutritional traits is valuable for precise and simultaneous genetic manipulations to expand the double benefit from both traits. A recent report showed that carotenoid extracts from various maize genotypes could efficiently inhibit the production of certain mycotoxins [35]. To efficiently exploit such unintended benefits from increased carotenoids in maize genotypes, understanding the underlying genetic mechanisms conditioning the desirable association of the two traits is critical.

3. Genomic-Enhanced Improvement

A wide array of studies on the genetics and genomics of plant carotenoid pathways [36] have generated tremendous knowledge which is being manipulated for accelerated development of biofortified crop plants. The molecular and biochemical dissection of the carotenoid biosynthetic pathway opened new avenues for accelerating the genetic improvement of carotenoid accumulation in maize [19,37,38]. Advances in genomics and associated new disciplines have immensely facilitated accelerated cultivar development. The three vital pillars of genomic-led breeding are the availability of rapid, inexpensive, and flexible genotyping methods, accurate and increased throughput plant phenotyping facilities, and innovative data acquisition, archiving, and analysis systems. Harnessing these enabling technologies paves the way for efficient assessment of the extent of genetic variability in an existing large number of genetic materials to develop maize cultivars biofortified with PVA.

The maize improvement program of IITA has thus integrated emerging Omics and ICT tools and techniques to enhance genetic gain [39]. In the revised breeding scheme, molecular tools are deployed at every stage of the breeding pipeline, starting with pre-breeding. The first and foremost step is to characterize the germplasm accurately.

3.1. Assessment of Genetic Variation of Carotenoids

In the conventional breeding of maize, exploitation of heterosis in breeding maize with enhanced carotenoid accumulation requires the identification of adapted and agronomically superior parents. Molecular markers provide additional information that could be used to better understand the genetic relationships among maize inbred lines, leading to more effective utilization of the inbred lines in the breeding programs for developing synthetic varieties and hybrids. Moreover, markers are invaluable in forming complementary heterotic populations from which promising inbred lines are extracted. The line development steps used routinely by breeders include estimating the combining ability of the inbreds for grain yield and other traits, grouping the inbreds, and identifying the best testers under contrasting environments. The first standard deployment of molecular markers was to assist in these processes. Parental selection based on the genetic diversity of yellow kernel lines [40] led to the development of productive hybrids with high PVA content and desirable agronomic performance [41]. In one of the fundamental areas of the pre-breeding stage, markers were successfully deployed to assess and identify suitable testers with desirable combining ability effects, to breed provitamin A-biofortified hybrid maize [42]. In addition to evaluating existing genetic variation in adapted germplasm, introducing exotic source germplasm is vital. In this vein, evaluation of many tropical-adapted orange and yellow endosperm maize inbred lines with diverse genetic backgrounds yielded a large number of inbred lines with increased provitamin content combined with desirable agronomic and adaptive traits. Temperate germplasm was used as a source of β -carotene and other carotenoids to elevate provitamin A content up to the current breeding target of $15 \mu\text{g g}^{-1}$ in tropical-adapted maize [43].

3.2. Population Improvement through MARS

Population improvement is one of the breeding strategies that aims to enhance the entire genetic base of the population through cyclical crossing and selection in successive generations with the ultimate goal of increasing the favorable allele frequency of the desirable trait. One of the approaches used to improve a population is marker-assisted recurrent selection (MARS). To enhance the PVA content of maize in two synthetic maize populations—heterotic group A [44] and heterotic group B (HGB)—two cycles of MARS increased the frequency of favorable alleles [45]. After the two cycles of selection, the content of β -carotene, PVA, and total carotenoids increased with selection cycles in HGA. In contrast, in HGB, only α -carotene increased with the selection cycle. β -cryptoxanthin increased at C1 but decreased at C2 in HGB. After two cycles of selection, the contents of β -carotene, PVA, and total carotenoids were improved by 40%, 30% and 36%, respectively, in HGA. The content of α -carotene and β -cryptoxanthin were enhanced by 20% and 5%, respectively, after two cycles of selection in HGB. The number of effective alleles and observed heterozygosity decreased with selection cycles, while expected heterozygosity increased at C1 and decreased at C2 in HGA. In HGB, the number of effective alleles observed, and the expected heterozygosity, increased at C1 and decreased at C2. In both populations, the fixation index increased after two cycles of selection. The most significant part of genetic variability was found within groups accounting for 86% of the total genetic variance in both populations. Generally, marker-assisted recurrent selection effectively improved PVA carotenoid content [45]. Not surprisingly, genetic diversity in the two synthetic populations declined after two cycles of selection. However, further multi-environment evaluations of the three cycles of the two synthetics in a hybrid combination showed genetic improvement per cycle of MARS in the parental synthetics, which ranged from 5% in carotene content to 26% for lutein [46]. MARS-based population improvement

demonstrated that breeders could shorten the time needed to develop outstanding inbred lines enriched with essential carotenoids. Some of the lines had twice the recommended kernel PVA, proving the effectiveness of the selection method, thereby enhancing genetic gain for carotenoid contents in the synthetics.

3.3. Forward Breeding Using Trait-Linked Molecular Markers

Implementing and accomplishing accelerated crop improvement in breeding programs relies on early-generation screening of breeding materials for desirable traits. Forward breeding signifies a rapid and low-cost genotyping alternative (platform) for improving the rate of genetic gain in breeding programs. Early-stage fixing of a desirable trait is achievable by selecting the breeding materials using SNP markers tightly linked to the trait of interest. Stimulated and supported by the High Throughput Genotyping (HTPG; <https://cegsb.icrisat.org/high-throughput-genotyping-project-http/> (accessed on 6 February 2024)) project, IITA recently initiated forward breeding for PVA and other traits. The carotenoid biosynthetic pathway in maize is regulated by polymorphisms associated with two key genes, *lcyE* and *crtRB1*, that control vital steps in the pathway. Using molecular markers derived from the functional polymorphism associated with these genes contribute to the rapid selection and development of PVA-enriched lines in breeding programs. The *crtRB1* gene encodes beta hydrolase 1, which underlies a principal QTL associated with beta-carotene levels in maize [47]. KASP assays for PVA, derived from nucleotide variants in the *crtRB1* gene, were designed and used for screening [48]. SNP markers for PVA are considered relatively accurate and widely used in selecting progenies featuring favorable alleles of the *crtRB1* gene. A total of seven *CrtRB1*-derived SNP assays were deployed. However, one of the seven SNPs, *snpZM0015*, is the most predictive marker and is located within a gene on chromosome 10 (Figure 2). Therefore, it will be cost-saving if users can simply use these markers instead of genotyping with all seven markers initially proposed for screening high PVA lines. The current genotyping platforms were set up so that a panel of 10 SNPs linked to various traits of interest can be assayed simultaneously in a single genotyping run. Depending on the population and the breeders' objective, the trait-linked markers can be combined flexibly (e.g., 7 PVA (pro-vitamin A) + 3 MSV (maize streak virus); 3 MLN (maize lethal necrosis) + 3 Striga + 4 PVA).

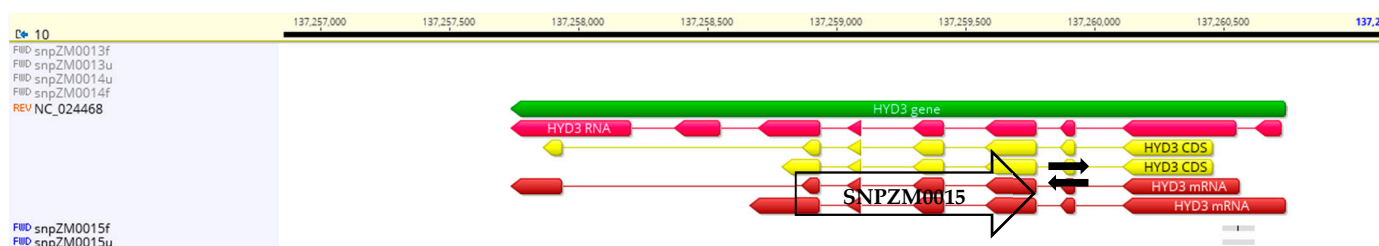


Figure 2. Position of snpZM0015 in the crtRB1(aka Hyd3) 3' TE region on chromosome 10.

These groups of samples were genotyped using the PVA-MSV and MLN-PVA SNP panels. Thousands of early-generation maize lines were genotyped using a panel of SNP markers linked to PVA and other adaptive traits, including MSV, Striga, and aflatoxin, beginning in 2017. For instance, in 2019, >1500 samples were genotyped using the MLN-PVA marker panel. Allelic data output of the PVA SNP snpZM0015 showed that 55.6% of the population were heterozygotes, 42.2% had the favorable allele, and 1.9% displayed the unfavorable allele for the marker. Selected early generation lines (i.e., S3 or S4) are subjected to further field evaluation for agronomic performance and productivity in multi-environment replicated trials.

4. Discovery and Validation of Markers

The prohibitive cost of laboratory analysis for carotenoid content calls for the identification and use of additional predictive markers to enhance genetic gain in PVA content

in maize. Marker-assisted selection (MAS) for PVA in maize can be quickly adopted in the genetic improvement of maize for nutrient enrichment. The elucidation of carotenoid biosynthesis pathways and the discovery of key genes in plants opened up the avenues for the identification of nucleotide variations that were utilized to develop functional markers for the selection of germplasms with higher carotenoid accumulations in maize [7]. Following the discovery of the key genes involved in carotenoid metabolism in temperate maize germplasm, the effectiveness of the various markers was assessed in diverse tropical maize germplasms at IITA [49,50], including the search for additional genes playing an important role in carotenoid accumulation [51]. The functional markers' predictive accuracy appeared variable depending on the genotype and the combination of favorable alleles emanating from the different genes [39]. The search for additional genes corroborated the previous findings, including *crtRB1* (chromosome 10), *lcyE* (chromosome 8), and *ZEP* (chromosome 2), affirming the significant effects of the two known major genes, namely *lcyE* and *crtRB1*. Although the association study detected significant novel associations with regulatory elements, such as several transcription factors and uncharacterized protein-coding genes, the role of these genetic factors remains to be investigated [51].

In another set of 108 inbred lines studied to validate the effect of the *Zep1*, marker *Zep*-SNP(801) was found to affect only α -carotene [50]. However, there was a trend that the stacking favorable alleles from all markers, including *crtRB1* and *LcyE*, within a genotype yielded higher PVA content.

Improved markers were developed and tested for efficiency using resequencing and comparative analysis. The optimization effort improved efficiency with respect to the *crtRB1* gene, while no considerable improvement was recorded in the *LcyE* gene. More advanced multi-omics approaches are being explored using transcriptome, proteome, and epigenetics [52].

In a more recent experiment, 64 tropical PVA biofortified maize inbred lines of temperate origin were assessed with the established markers linked to PVA. The key finding in this assessment was that lines carrying the favorable alleles of *LcyE* exhibited the highest concentrations of non-PVA carotenoids, namely, lutein and zeaxanthin, while the lines carrying the *crtRB1* alleles were rich in PVA carotenoids, namely, α -carotene, β -carotene, and β -cryptoxanthin [29]. Further studies are, however, needed to assess the consistency of these results. Given that both PVA and non-PVA carotenoids have health benefits, developing and validating markers specific to the two categories of carotenoids will be necessary. The repertoire of molecular markers associated with nutritional quality traits, driven by the declining cost of marker genotyping, has enabled effective and efficient integrated molecular and conventional breeding workflow [7].

5. Prospect and Perspective

Molecular breeding schemes for developing nutritionally enriched crops offer a cost-effective and efficient alternative to circumvent the prohibitive cost of phenotyping for nutritional traits [6,7]. Multi-omics-driven discovery of the genetic factors underlying the vast array of nutritional traits is advancing earnestly. Various advanced Omics methods, from nutrigenomics to gene editing, have been tailored to elucidate biochemical pathways underlying essential macro and micronutrients. A thorough understanding of the regulatory control of carotenoid accumulation, including biosynthesis and degradation, is expected to open new opportunities [19]. Trait-linked markers, developed based on the biosynthesis pathway of many vitamins and micronutrients, have enabled the breeding process to develop single- or multiple-trait biofortified maize cultivars.

The ever-growing evidence and knowledge regarding carotenoid metabolism in flowering plants, in general [19,53], and in cereal crop plants, in particular [7,54,55], stimulated further genomic and genetic investigations. Due to the conservation of genes involved in carotenoid biosynthetic pathways, comparative genomics can be exploited to transfer knowledge from one species to another. The recent momentum in elucidating the metabolism of micronutrients should be expanded to novel breeding targets as well as to

the simultaneous enhancement of nutritional qualities in staple food crops. The current focus on PVA should be complemented with the enhancement of non-PVA carotenoids (zeaxanthin and lutein), other vitamins (Vit B and E and antioxidants—anthocyanin), antinutritional factor phytate, and minerals other than Fe and Zn.

In light of the affordable genotype cost and the availability of predictive markers for key nutritional quality traits, it is high time to mainstream breeding for quality traits in the product profile or breeding pipeline of maize to fast-track the introgression and/or pyramiding of favorable alleles into elite maize lines. However, there is a crucial need to validate the markers in maize lines of tropical origin. Equally important is the development of robust predictive markers for non-PVA quality traits to simultaneously improve multiple traits. The importance of micronutrient deficiency in SSA cannot be overemphasized. It is vital to pay equal attention to genetic improvement of quality traits, productivity, and stress tolerance.

The aforementioned new technologies can be integrated with the existing methods to further fast-track the development of nutrition-dense maize cultivars. The potential of genome editing in curbing nutritional deficiency through crop biofortification is emerging. For instance, the use of genome editing to alter genes involved in the carotenoid pathways in bananas and tomatoes was described [56]. The critical first step for genome editing is the identification of target genes. Several genes controlling nutritional quality traits, including carotenoids, minerals such as Fe and Zn, phytic acid, quality protein maize, and others have been reported. Once reliably validated using advanced functional Omics methods, these genes can be successfully modified by CRISPR/cas or other gene-editing techniques in superior elite lines [7,55–57].

Agronomic biofortification, which involves the foliar application of crucial elements to augment the micronutrient content of the soil, helps modify a crop's nutrient profile. Integrating genetic advancement with agronomic biofortification complements the global effort to end malnutrition in low-income countries [24,58–60].

Nutrigenomics, a new branch of science investigating the interactions between nutrition/diet and an individual's genetic profile, provides a better understanding of how genetic variations influence nutrient metabolism and requirements. Given the widespread nature of food and nutrition insecurity, the potential use of nutrigenomics may seem far-fetched and low priority, but it may affect a considerable proportion of the population [61].

6. Conclusions

The prevalence of nutritional insecurity remains a hindrance to achieving Sustainable Development Goal-2 (SDG-2), and it is more prominent in SSA. There are many reports of the pervasiveness of food insecurity in Africa [6,62,63]. The food security challenge is compounded by inadequate nutritional security resulting in dire health situations that call for effective mitigation endeavors [2,64].

Complementary to enhancing crop productivity, it is high time to envisage comprehensive and integrated solutions to eradicate hidden hunger by harnessing emerging genetic improvement and agronomic approaches to surmount the dire health situation faced by the millions of low-income households who lack access to a balanced diet, supplements, or fortified foods [6,62,63]. The resultant nutrient-dense grain can reduce the impact of non-infectious and preventable diseases in a population that depends on maize as a staple food. After biofortification, factors that cause postharvest nutrient loss, such as traditional processing, agronomic practices, and storage condition, should be considered to sustainably furnish the recommended level of nutrients.

In the face of expensive, laborious, and low-throughput phenotyping, developing tightly linked markers for selecting genotypes with higher concentrations of various nutrients is vital. As a prerequisite, novel genes involved in the accumulation of different micronutrients in maize can be identified by multiple approaches, including QTL mapping, association analysis, and other methods. Subsequently, the candidate genes/loci are sub-

jected to functional validation studies involving multi-omic strategies. The outcomes will be a robust marker assay for breeding or reliable targets for metabolic engineering.

Improvement of complex traits, such as micronutrients, requires understanding not only of nutrient accumulation but also degradation, interaction with other factors, such as phytate, and postprocessing loss. Harnessing new technologies and approaches to enhance crop nutritional quality obviously calls for a mutual effort from a multi-disciplinary team comprising conventional breeders, biotechnologists, agronomists, biochemists, molecular biologists, nutritionists, and others.

Notably, projects/campaigns aiming at alleviating malnutrition in Africa have, often, focused on vital nutritional challenges such as VAD. In an environment where health concerns are significant, amelioration of deficiency in a single nutrient could be undermined by the impact of other deficiencies. Therefore, simultaneous improvement of multiple quality traits, where tools and resources permit, is imperative. The advent of multi-omics and cognate technologies is poised to make such actions achievable in a relatively shorter time and in a more-precise manner.

In the wake of remarkable advances in life sciences, ranging from multi-omics, genome editing, speed breeding, genomic predictions, bioinformatics, phenomics, and others, it is an opportune time to mainstream biofortification, as elaborated recently [65], in the future development of every new maize cultivar. Mainstreaming biofortification implies augmentation of nutritional quality traits into the existing released stress-resilient genotypes, such as disease resistant, Striga tolerant, and drought and heat-stress tolerant materials.

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