

Review

## Genetic Enhancement of Early and Extra-Early Maturing Maize for Tolerance to Low-Soil Nitrogen in Sub-Saharan Africa

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### ABSTRACT

To increase and improve food crop production and crop management for sustainable agricultural development, the Maize Improvement Program (MIP) of the International Institute of Tropical Agriculture (IITA) has, in partnership with national and international organizations, focused attention on the genetic improvement of maize (*Zea mays* L.) for tolerance/resistance to abiotic and biotic stresses constraining maize grain production. One of the abiotic stresses into which new technologies have been established for crop protection and sustainable natural resource management is low soil nitrogen (N). Nitrogen, an important plant nutrient required for growth and productivity, is not readily available and little quantity or none is applied by farmers for maize production. Because of long periods of bush fallow, the absence of N was not noticeable at the initial stages of maize production in West and Central Africa (WCA). However, with the fallow period gradually reducing and totally disappearing, it has become imperative for external supply of N in maize production. Taking a cue from the International Maize and Wheat Improvement Centre (CIMMYT), IITA has been breeding low-N tolerant maize and several low-N-tolerant maize varieties and hybrids are now available to farmers. Reviewed in this manuscript are the efforts used to develop low-N tolerant early and extra-early maturing maize by the MIP of IITA. The review covers the objectives, methodology, and output of the

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research, including the genetics of tolerance, ongoing conventional and molecular approaches, and the gaps that new research could fill.

**KEYWORDS:** low soil nitrogen; tolerance; maize; genetic enhancement; *Zea mays* L

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## INTRODUCTION

### Review Methodology

Although research into low-N tolerance in maize is relatively new in SSA, a lot of information has been accumulated by different researchers. This review covered the research conducted by international research institutes, particularly IITA in WCA and CIMMYT in Eastern and Southern Africa (ESA), and national programs, especially universities and national research programs. We focused on how the research was carried out, highlighting their conclusions, and pinpointing what is missing that new research may address. Our focus was also on the coverage and contribution to the genetic improvement of low-N tolerance in maize, comparing the research findings of one group with those of another. Furthermore, the empirical evidence and new research methods as they pertain to low-N tolerance research and its wider subject matter were thoroughly and critically scrutinized. To be useful to other researchers and practitioners, we clearly stated the objectives of what we needed to obtain from the reviewed studies, made the methodology explicit, assessed the validity of the findings of the studies, and made a systematic presentation and synthesis of the findings of each study in the review. We accomplished all of these by searching through the existing literature, especially from IITA and CIMMYT in the last 20 to 30 years. Therefore, the review covered conventional and ongoing molecular aspect of low-N tolerance research in early and extra-early maturing maize.

### General Overview

Maize (*Zea mays* L.) introduced to WCA more than 500 years ago, has become fully adapted to the region, producing well over 11 million metric tons of grain annually. It is cultivated in mixed crop situations over a large area and, to a smaller extent, in pure stands, usually mechanized. Initially, under the mixed cropping system, maize cultivation was under bush fallow for several years, but with the increase in population and urbanization, the period of fallow has gradually reduced and, in many cases, has disappeared and the maize crop must be grown annually in the same field. The maize crop thrives and produces high yields under high fertility conditions which, to a limited extent, is supplied through the fallow system. However, with the fallow system disappearing, it has become necessary to supply inorganic fertilizers, particularly nitrogen (N), the nutrient most needed by the crop. Unfortunately, the WCA maize farmers apply less than 10 kg N per hectare (ha) to the crop thus keeping

the grain production rather low, regardless of the type of variety cultivated and the agronomic practices applied. Apart from the fact that ideal fertilizers are hard to come by, most fertilizers available are adulterated, a high proportion of the real nutrient is lost through volatilization, farmers cannot afford the capital to purchase them, and the timing of fertilizer application is usually missed. All of these and perhaps many more are the reasons for the low rates of fertilizers being applied.

The Maize Improvement Program (MIP) at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria initiated a breeding program for low soil N tolerant early and extra-early maturing maize several years ago. A few years before then, CIMMYT scientists had initiated a similar program in Mexico and later brought it to Kenya to cover ESA countries. Together, all countries of Eastern, Southern, Central, and Western Africa are well covered in the research for improved tolerance to low-soil N, and their outputs are reviewed in this report.

### **Nitrogen Dynamics in African Soils**

Nitrogen is an essential nutrient required for healthy plant growth and development. It is an important constituent of chlorophyll, which plays an important role in photosynthesis, a major requirement for high grain yield of maize. Nitrogen plays a significant role in plant metabolism, and it is also an important constituent of protein synthesis and nucleic acids [1]. The lack of nitrogen at the physiological stage in maize reduces leaf area development, photosynthetic rate, increases kernel and ear abortion, accelerates leaf senescence, and reduces crop kernel weight [2,3]. Tropical soils in most developing countries of SSA are low in available nitrogen and organic matter content, resulting in the cultivation of maize on nitrogen-depleted soils. The edaphic condition of the nitrogen-depleted soil is further aggravated due to the subsistence level of resource-poor smallholder farmers who constitute a large percentage of maize producers and can hardly afford the high cost of mineral fertilizers to supplement the available nitrogen in the soil. Consequently, maize production in the sub-region is seriously affected by inadequate use and high cost of nitrogen-based fertilizers or non-availability of fertilizers, or lack of funds for farmers to buy fertilizer [3–5]. A study by Ertiro et al. [6] reported a 71% yield reduction under severe low soil nitrogen conditions. Similarly, Obeng-Bio et al. [7] reported a yield reduction of about 80% under low nitrogen conditions. Therefore, there is a need for the development and commercialization of superior maize germplasm with tolerance to low soil nitrogen to ensure increased maize productivity and sustained maize production as opposed to the practice of long fallow by smallholder farmers in SSA. High-yielding nitrogen-use efficient maize genotypes under low soil nitrogen will provide resource-poor farmers in depleted soil areas with tolerant improved maize varieties to achieve better yield [8–10]. Unfortunately, N is the most limiting nutrient in tropical soils, especially the savannas of WCA, an otherwise highly productive

environment for maize due to the high incoming solar radiation, reduced incidence of pests and diseases, low humidity, and low night temperature [3]. The dynamics of the amount of nitrogen in the soil and its availability to plants are greatly influenced by genetic and environmental factors. The amount of nitrogen available to plants is dependent on the level of soil organic matter and clay content; both fix nitrogen in the soil and can therefore release the nutrient (mineralization) under some conditions [11,12]. The soils of WCA contain low levels of organic matter and the low fixation type, which is clay is largely kaolinite. Nitrogen applied as fertilizer is an additional way of making nutrient available in the soil [3]. Nitrogen losses through running water, volatilization, leaching, and plant uptake—all significant environmental factors—reduce the amount of soil nitrogen that is available. Genotypic differences are also known to exist in the ability of plants to take up nitrogen in the soil [13]. Plants also differ in their ability to mobilize photosynthates produced, using absorbed nitrogen as input, into the economic part, and relocation of assimilates during conditions of nitrogen stress [11]. Although maize germplasm can be screened for each of these mechanisms using appropriate indices, screening based on yield and associated secondary traits under low nitrogen offers the prospects of identifying genotypes that owe their superiority to a combination of the mechanisms for low-nitrogen tolerance [14]. Logrono and Lothrop [15] estimated that loss of maize yield due to low-N stress varies from 10 to 50% per year in SSA while the occurrence of the stress during grain-filling accelerates leaf senescence and reduces crop photosynthesis and kernel weight [2]. Leaching and the extensive removal of crop residues for animal feed and fuel have compounded soil N depletion in the soils of SSA [16]. Weber et al. [5] indicated that the maize plant requires 50–60 kg N and 30 kg P ha<sup>-1</sup> in plant-available forms for 1 ton of grain produced. There is a high correlation between N requirement and maize grain yield [2,17,18]. Low-nitrogen effects could be reduced through the application of organic manure and inorganic fertilizer and the use of legumes capable of fixing atmospheric nitrogen, but only a few farmers can afford enough good manure for application [19,20]. One possible alternative is to use nitrogen-fixing legumes in rotation, but the time required to grow the legumes prevents the acceptance of this alternative by farmers [21]. Therefore, breeding for tolerance to low-N offers the most economic and sustainable approach for increased maize yields by smallholder farmers who utilize low agricultural inputs in SSA. Low-N tolerant cultivars are superior in the utilization of available N, either because of enhanced N-uptake capacity or more efficient use of absorbed N for grain production [22]. Therefore, the development and commercialization of maize germplasm with enhanced tolerance to low-N are essential prerequisites to sustain increased maize productivity in the region [22]. Bänziger et al. [23] found that improvement for drought tolerance also resulted in improved performance under low-

N conditions, suggesting that tolerance to either stress involves a common adaptive mechanism.

The adoption of high yielding and nitrogen use efficient maize genotypes provide a great opportunity to reduce the surging cost of fertilizer [3]. Moreover, to support the rapidly expanding population and to motivate farmers who typically apply moderate levels of N to their maize fields, genotypes with high yield potential under low-N conditions are needed. Smallholder maize farmers in the stress-prone areas of WCA will greatly benefit from genetic improvement strategies that aim at tailoring maize for better performance under low-N [9]. In addition to improved yield under severe N-deficient conditions, these cultivars will be more responsive to the small N doses that farmers apply. Improved maize cultivars for low-N conditions have been developed using a variety of breeding techniques, including selection for increased yield under N stress and for specific mechanisms that are confer low-N tolerance [24]. In a study by Castleberry et al. [25], larger selection gains for enhanced grain potential were obtained in high-N conditions compared to low-N ones. Nonetheless, it is anticipated that genetic gains under low-N will be higher when selection is done under both low and high-N. Furthermore, several studies have reported an outstanding performance of tropical maize genotypes selected for drought tolerance under low-N conditions [15,23,24,26-28]. Selection for *Striga* resistance under low-N could also result in a concomitant increase in tolerance to low-N. For example, Badu-Apraku et al. [29] evaluated three cycles of S<sub>1</sub> progeny selection for *Striga* resistance in the extra-early maturing white population, TZEE-W Pop STR under low-N (30 kg N ha<sup>-1</sup>) and the recommended high-N (120 kg N ha<sup>-1</sup>) rates and found that the gains in grain yield were higher under low-N than high-N. As a result, selection under low N has become an important strategy of the IITA Maize Improvement Program (IITA-MIP) for developing low-N tolerant cultivars.

Low-N stress in SSA is caused by several factors, including the widespread removal of crop residues for use as animal feed and fuel, and leaching of soil N below the root zone due to heavy rainfall [23]. Additionally, poor weed control in farmers' fields increases the incidence of N deficiency, which is worsened by the application of sub-optimal levels of inorganic fertilizer due to high prices and the unavailability of fertilizer [30]. Nitrogen is the most limiting nutrient in tropical soils. Consequently, lack of or low-N availability occurring early in the life of the plant reduces plant leaf area expansion, as well as photosynthesis [23]. Nutritional stress occurring just before flowering causes severe damage to structures that determine yield. The low level of nitrogen in tropical soils is a constraint to high productivity if fertilizers, either organic or inorganic, are not adequately applied. Available nitrogen is reduced by losses through running water, volatilization, leaching, and nitrogen taken up by plants. Estimated yield losses due to N stress alone range between 1 and 50% [31].

### **Breeding Maize for Tolerance to Low Soil Nitrogen at IITA**

To alleviate the effects of low-N stress, the IITA-MIP has embarked on the development of low-N tolerant genotypes during the last three decades, resulting in the development of several low-N tolerant populations, inbred lines, and hybrids [3]. The availability of extra-early and early maturing maize in the savannas of SSA has boosted maize production and consumption either as green maize or dry grain and has helped to fill the hunger period in July in the savannas of the sub-region, during which other food reserves are depleted due to the long dry period [32]. Additionally, the availability of extra-early and early maturing maize hybrids has resulted in considerable increases in maize productivity and production, leading to remarkable improvements in the incomes and well-being of farmers in the sub-region [3]. Despite the remarkable progress that has been made, low soil nitrogen, drought, and *Striga hermonthica* parasitism are still major limitations to maize production and productivity in SSA. Therefore, to mitigate the effects of low-N stress, the IITA-MIP has embarked on the development of low-N tolerant, open-pollinated, extra-early, and early maturing cultivars and hybrids that can utilize available nitrogen more efficiently. Several maize genotypes with good nitrogen use efficiency have been identified following several years of extensive testing in SSA and have been used as parents to develop adapted source populations for improvement using recurrent selection methods. Several promising extra-early and early maturing hybrids and open-pollinated varieties with high grain yield under low nitrogen have been identified and commercialized in SSA.

### **Screening Methodologies for Low Soil Nitrogen Tolerance**

Because of the existence of genotype  $\times$  environment interaction (GEI) in most yield trials, attention is presently being focused on breeding for tolerance to low-soil N in SSA. Genotypes that optimize grain yield under optimal soil nitrogen are not necessarily the best when soil nitrogen is limiting. Many maize farmers in SSA do not apply fertilizers or apply them in quantities too low for the full expression of the yield potential of the varieties that are cultivated; therefore, breeding for low-N tolerance is the most economically feasible and sustainable option for increased grain production [3]. It is desirable to have genotypes that can show superiority under both stress and optimal soil nitrogen conditions. Evaluation for tolerance to low-N must, therefore, be conducted under both low-N and optimal growing conditions. One problem faced by maize breeders is the determination of the low-N level under which screening would be carried out. The problem is aggravated by the differences in soil types and solar radiation received in the different agro-ecological zones in SSA. As a starting point for successful screening for tolerance to low-N in SSA, the N level in the soil should be well below 1.5 g/kg, the critical level above which no fertilizer is required for optimum yield of maize. Screening of maize

genotypes for low-N tolerance is conducted on experimental plots which have been depleted of soil nitrogen by continuous planting of maize and removal of the stover after each harvest. Soil samples from low-N plots are analysed to ascertain the amount of available N in the soil and to estimate the additional amount of N required for the set low-N level (e.g., 30 kg of N ha<sup>-1</sup>). The soil of the site under which maize genotypes are screened must be uniform to reduce experimental error that could be associated with identifying genotypes that truly owe their superiority to the ability to excel under conditions of nitrogen stress. Measures that are used to increase precision in non-stressed environments will also improve precision in nurseries for breeding for low-N tolerance [3].

Screening for tolerance to low-N involves the exposure of the genetic materials to at least two contrasting levels of N fertilizer, e.g., 30 and 90 kg N ha<sup>-1</sup> with the lower level serving as the testing rate, while the higher level serves as the control. The two levels are used for selection to ensure that selected low-N tolerant genotypes are not necessarily mediocre in performance under high-N. Soil tests are carried out and inorganic N fertilizer added to make up the two levels. In our early and extra-early trials, two sites are used for low-N screening: Ile-Ife in the forest agroecology and Mokwa in the southern Guinea savanna (SGS). Soil test results showed that the soils at these sites have significantly been depleted of its inherent N. In addition to the specific low-N screening sites, the *Striga* screening sites at Mokwa and Abuja also serve as indirect screening sites for low-N because only 30 kg N ha<sup>-1</sup> is applied to the *Striga*-infested plots, while the non-infested plots that receive optimal recommended N rate (90 kg N ha<sup>-1</sup>) serve as the control. Recommended measures are employed for the control of weeds, insect pests, and diseases. Border rows are essential and often included in all trials. For extremely poor soils, yield may approach zero. In such soils, some little quantity of nitrogen fertilizer, such as 30 kg ha<sup>-1</sup> or less, may be applied prior to the N treatments [3,33,34].

Apart from grain yield which is the primary trait considered in most maize breeding programs, several other agronomic traits were assayed when screening for low-N tolerance, including flowering (days to 50% anthesis and silk emergence), plant and ear heights, plant and ear aspects, root and stalk lodging, leaf senescence, and ears per plant. Anthesis–silking interval (ASI) and a few other traits are generated from the primary traits that are measured [14,23,26,33–35]. Heritability of grain yield is low under stress factors; therefore, selection for grain yield alone without some other suitable secondary traits may be ineffective under low-N conditions. Pollen shed and silk emergence are usually delayed under nitrogen stress, with silk emergence exhibiting a greater delay resulting in higher ASI. Genotypes with low ASI values under N stress are considered tolerant to low soil nitrogen. Desirable secondary traits are those that have high heritability and significant genotypic and phenotypic correlations with grain yield under nitrogen stress [3,33,35]. Therefore, to improve the efficiency of selection for low-N tolerant genotype, secondary traits such

as ears per plant, delayed leaf senescence, and anthesis-silking interval are often used in combination with grain yield [23,26]. Germplasm for tolerance to low-N can be screened from diverse sources, including open-pollinated varieties (OPVs) from farmers' fields, elite populations, different types of families (such as  $S_1$ , full sib and half-sibs), inbred lines, and all types of hybrid varieties. In WCA, OPVs from farmers' fields are sources of genes for tolerance to low soil N because farmers grow their maize with little or no fertilizers [3]. By selecting high-yielding plants under such conditions as sources of seeds for the next cropping season, they inadvertently may have increased the frequency of genes for tolerance to low-N in their populations. Also, recurrent selection could be deployed for genetic improvement of elite populations for enhanced tolerance to low-N. Subsequently, inbred lines could be extracted from the improved low-N populations.

Breeders at IITA and some West Africa (WA) national programs have used recurrent selection to improve grain yield and resistance/tolerance to abiotic and biotic stresses of maize for about four decades [29,36–43]. Breeders initially deployed the International Progeny Testing Trial (IPTT) strategy designed at CIMMYT. A critical requirement for the IPTT strategy, is availability of data from a cycle of selection spanning two years and two seasons each year [36]. Despite being effective, the IPTT approach was too resource-intensive for national programs, and the data obtained from some sites were sometimes highly variable and thus unfit. As a result, the number of testing locations was decreased to four, all of which were managed by IITA, with three replications at each location. Recurrent selection has also been deployed for newly developed populations. For example, Menkir and Kling [38] evaluated the genetic gain in a genetically broad-based, late-maturing (about 120 days to physiological maturity) maize composite, TZL COMP1-W, which had undergone six cycles of recurrent selection. They found that selection improved performance by 24% cycle<sup>-1</sup> and ears per plant (EPP) by 9% cycle<sup>-1</sup>. Following three cycles of full sib recurrent selection for improved tolerance to low-N, Alabi et al. [41] found that each cycle of selection in a low N tolerant pool, dubbed LNTP-Y resulted in grain yield improvement of 0.15, 0.20 (5.4%) and 0.13 t ha<sup>-1</sup> (4.1%) under high N, low-N and across N levels, respectively. Yield performance of  $C_3$  under high-N was lower than those of  $C_1$  and  $C_2$  resulting in a nonsignificant gain from selection when the cycles were evaluated under high-N (120 kg ha<sup>-1</sup>). Recurrent selection has been used to improve early and extra-early maturing maize varieties as well. Salami et al. [39] evaluated the progress from two cycles of reciprocal recurrent selection in two early maize populations, TZE Comp3 and TZE Comp4. Selection gain in grain yield per cycle was  $0.29 \pm 0.05$  t ha<sup>-1</sup> (10.4%) for TZE Comp3 and  $0.17 \pm 0.05$  t ha<sup>-1</sup> (5.7%) for TZE Comp4. Results from an earlier evaluation of the selection program showed mid-parent heterosis of 4% for  $C_1$  and 7% for  $C_2$  relative to the original population cross [37]. More extensive recurrent selection studies have been carried out in two other



early (TZE-W Pop DT STR C<sub>0</sub> and TZE-Y Pop DT STR C<sub>0</sub>) and two extra-early (TZEE-W Pop DT STR and TZEE-Y Pop DT STR) populations by IITA scientists. From each population, 167 S<sub>1</sub> lines were generated and evaluated along with two checks in Ferkessédougou (Ferke) under artificial *Striga* infestation; Sinématiali (Siné), a high-yielding, non-stress environment; and Kamboinse, a drought stress environment, all in Côte d'Ivoire. In 1998, the first cycle of improvement in each population was completed by inter-crossing the top 25–30% families identified in 1997 through progeny yield trials. Thereafter, each population was taken through three more cycles of S<sub>1</sub> recurrent selection under *Striga*, drought, and non-stress environments, using 196–256 S<sub>1</sub> lines and, with a selection intensity of 25–30%, the top families of each population were recombined to reconstitute each population for the next cycle of selection. Evaluation of the four cycles of recurrent selection products in several stress and non-stress environments showed that grain-yield response in the two early populations under *Striga* differed greatly: about 70.6 kg ha<sup>-1</sup> (6.3%) cycle<sup>-1</sup> for TZE-Y Pop DT STR and 352.5 kg ha<sup>-1</sup> (58.0%) cycle<sup>-1</sup> for TZE-W Pop DT STR. Corresponding values for the two populations under *Striga*-free environments were similar: 194.0 kg ha<sup>-1</sup> (6.6%) cycle<sup>-1</sup> and 186.5 kg ha<sup>-1</sup> (6.0%) cycle<sup>-1</sup>, respectively. Similarly, ASI decreased in the improved cycles relative to the C<sub>0</sub>. For the extra-early populations, genotypes, locations, and years significantly affected grain yield, days to silk, ASI, and ears plant<sup>-1</sup> under both *Striga*-infested and *Striga*-free conditions. Under *Striga*-free conditions, these sources of variation also significantly impacted plant height and stalk lodging. Genotype mean squares were also significant for stalk lodging as well as *Striga* damage, but not for the number of emerged *Striga* plants at 8 and 10 weeks after planting. Apart from year × location mean squares that were significant for seven of the ten traits, genotype by environment interaction effects were not significant in *Striga*-infested environments.

In the IITA Maize Program, selection for *Striga* resistance is normally done under low-N conditions without deliberate selection for low-N tolerance. Based on the improved germplasm and breeding techniques, selection for enhanced resistance to *Striga* and tolerance to drought began independently in 1988 and 1994, respectively, and have gone through three breeding eras. Several comparisons of older and newer hybrids under contrasting N levels have been reported [25,44–46], but similar studies in tropical maize are limited, thus making it difficult to completely ascertain the genetic gain that has been made for grain yield in relation to N fertility in the numerous varieties that have been released in SSA. For example, Castleberry et al. [25] evaluated 25 open-pollinated and hybrid maize cultivars used between the 1930s and 1980s and found that genetic gains for grain yield under low and high soil fertility were 51 and 87 kg ha<sup>-1</sup> year<sup>-1</sup>, respectively. Similarly, with four hybrids representing Brazilian maize germplasm released between the 1960s and 1990s, the most recent hybrid produced higher grain yields at all levels of N [45]. Also, a newer

hybrid (Pioneer 3902, released in 1988) produced approximately 25% more grain than an older hybrid (Pride 5, released in 1959) at both low-N and high-N [43]. In contrast to these studies, O'Neill et al. [46] showed that a hybrid widely grown in the USA during the 1970s (B73 × Mo17) produced approximately 8% more yield under the deficit N treatment than hybrids released in the early and late 1990s, while the latter had greater yield responses to applied N fertilizer. A series of studies were also conducted by IITA scientists to test the hypothesis that tolerance to low-N had been significantly improved while selecting for *Striga* resistance during the three breeding eras. The 50 early maturing cultivars used for the drought and *Striga* studies were also evaluated in replicated field trials in 2010 and 2011 at Mokwa, in the southern Guinea savanna and Ile-Ife in the rainforest agroecology under both low-N (30 kg N ha<sup>-1</sup>) and high-N (90 kg N ha<sup>-1</sup>) levels. The data were subjected to ANOVA and regression analysis. Grain yield increased significantly from the first to the third breeding era under both low and high-N (Table 1).

**Table 1.** Minimum, maximum, and mean ± se of grain yield (t ha<sup>-1</sup>) of maize cultivars of three breeding eras evaluated under low- and high-N conditions at Mokwa and Ile-Ife in 2010 and 2011.

Era	No. of varieties	Low-N			High-N		
		Min	Max	Mean ± se	Min	Max	Mean ± se
1 (1988–2000)	15	0.8	4.2	2.3 ± 0.056	0.9	3.2	3.2 ± 0.174
2 (2001–2006)	16	0.3	5.4	2.4 ± 0.063	0.8	5.8	3.3 ± 0.076
3 (2007–2011)	19	0.7	5.6	2.5 ± 0.055	1.0	5.5	3.7 ± 0.068

Similarly, for both low and high-N, plant and ear heights increased slightly, while ear aspect decreased from era 1 to era 3. Days to flowering, and stalk and root lodging were not affected by breeding era. Instead, in era 3 compared to era 1 and under low but not high-N, plant aspect and the stay-green character improved. In the ANOVA, the variety-within-era source of variation was highly significant for both low- and high-N environments. The highest-yielding cultivars under both N environments, such as TZE-W DT C<sub>2</sub> STR, EV DT-W 2008 STR, 2009 DTE-Y STR Syn, and TZE-W DT C<sub>1</sub> STR, were mainly from breeding era 3. For the 50 varieties, yield performance in the low-N environments predicted grain yield under high-N accurately, with an R<sup>2</sup> value of about 0.54. Analysis on individual era basis, however, showed that grain yield in low-N environments for eras 2 and 3 cultivars predicted grain yield in high-N environments than era 1 cultivars. Based on the results of this study, it was concluded that selection for *Striga* resistance and drought tolerance in early-maturing maize populations enhanced low-N tolerance in the maize cultivars derived from the populations. Recent breeding eras in WA showed greater improvement than previous eras.

No direct comparisons of grain-yield potential and other agronomic traits have been made across multiple stresses for the early-maturing, drought-tolerant, and *Striga*-resistant maize cultivars developed during

the three breeding eras. Since drought, *Striga* infestation, and low-N do not occur singly but occur together under field conditions, a holistic evaluation of the gains from the efforts over the three eras seemed warranted at this time to serve as the basis for the direction to pursue in future breeding efforts in the sub-region. Therefore, a study was conducted at 16 locations in WCA for 2 years to determine genetic improvement in grain yield of maize cultivars developed during three breeding periods, across drought, *Striga* infestation, low soil nitrogen, and optimal growing environments. The objective was to evaluate the genetic yield gain that had been made in breeding stress-tolerant early-maturing maize during the three breeding eras across multiple stresses (managed and natural drought stress, *Striga* infestation, low-N) and optimal growing environments. Grain yield had an annual genetic gain of 1.52% and 1.24% under multiple stresses and optimum growing environments, respectively (Table 2).

**Table 2.** Mean grain yield ( $\text{kg ha}^{-1}$ )  $\pm$ SE for three breeding Eras, regression parameters and percent genetic gain  $\text{Era}^{-1}$  for early maturing maize cultivars evaluated in two groups of environments in Benin, Ghana, and Nigeria, 2010 and 2011.

Environment	Era			Regression parameters			% Genetic gain $\text{Era}^{-1}$
	1 (15cvs)	2 (16cvs)	3 (19cvs)	$R^2$	Intercept	b-value	
Multi-stress (16 env)	2176 $\pm$ 54.20	2286 $\pm$ 49.20	2606 $\pm$ 49.00	0.93	1926.0	215.0	11.16
Non-stress (35 env)	3398 $\pm$ 52.50	3615 $\pm$ 46.70	3957 $\pm$ 42.30	0.98	3097.7	279.5	9.02
Mean	2760.25	2908.5	3206.5	0.96	2512.2	223.1	8.88

Note: Era 1: 1988–2000; Era 2: 2001–2006; Era 3: 2007–2010.

Across stress conditions, genetic gain in grain yield was  $30 \text{ kg ha}^{-1} \text{ year}^{-1}$  while an annual increase in grain yield of  $37 \text{ kg ha}^{-1}$  was recorded across optimum growing environments. The genetic gains in grain yield per annum were 0.56%, 1.52% and 1.62% for the cultivars of period 1, 2 and 3, respectively. Under stress conditions, only three agronomic traits; ears per plant ( $0.32\% \text{ year}^{-1}$ ), ear aspect ( $-0.51\% \text{ year}^{-1}$ ), plant aspect ( $-0.24\% \text{ year}^{-1}$ ), and days to anthesis ( $0.11\% \text{ year}^{-1}$ ) were significantly altered ( $p < 0.05$  or  $< 0.01$ ) during the three eras. The increase in grain yield from the first- to the third-generation cultivars across stress environments was associated with significant improvement in plant and ear aspects, increased ears per plant, and stay-green characteristic. Under growing conditions, there was a 1.24% annual increase in grain yield from first to third generation cultivars, and this rise was correlated with significant improvements in plant and ear heights, plant and ear aspects, husk cover, and the number of ears per plant. It was concluded that substantial improvement in the yield potential of maize had been made in this sub-region by breeding for stress tolerance during the past three decades. The

most productive and resilient cultivars in a range of stress conditions were EV DT-W 2008 STR, 2009 DTE-Y STR Syn, and TZE-W DT C2 STR, all from the most recent breeding era. These varieties should be promoted to help this subregion achieve food security. Recurrent selection had been effective in improving early and extra-early maize populations in WCA for tolerance/resistance to *Striga hermonthica* infestation, drought, and low soil N. The  $R^2$ -value (0.54) obtained by regressing yield performance in the low-N environments on grain yield under high N suggests that greater improvement would result if selection was carried out for N tolerance per se.

### **Strategies for Improving Effectiveness of Selection for Tolerance to Low-N in Early and Extra-Early Maturing Maize**

Grain yield under stress and optimal growing conditions is controlled by different physiological mechanisms [3,47,48]. Therefore, to achieve the highest expression of genetic variation for target traits that could be exploited in stress tolerance breeding programs, genotype evaluations should be conducted in an environment best suited to the specific agricultural ecosystems [2], which will allow for maximum production in that target environment [49]. To improve maize grain yield under low-N conditions, indirect selection under low-N is very important as this strategy accelerates the gains from selection for grain yield through the exploitation of specific adaptation. The development of high-yielding and stable maize hybrids with tolerance to low-N is crucial for increased maize productivity and offers the most economic and sustainable approach for increased maize yield by smallholder farmers in SSA who utilize low-N inputs. In maize breeding, genotypes possessing the most desirable traits such as high grain yield and earliness under optimal growing conditions are targeted. However, genotypes selected for high performance in high-input conditions often do not maintain those same high yields under low-input conditions partly due to lack of natural genetic variation of traits advantageous in stressful environments [50]. According to Bertin and Gallais [51] and Presterl et al. [52], the N uptake efficiency contributes more to NUE variation under low and high-N availability, while N utilization efficiency contributes more under low-N conditions. There is a wide variation within maize germplasm in terms of nitrogen nutrition particularly for low-N tolerance and NUE [26]. Genotypes with a short ASI and prolific genotypes seem to have the ability to remobilize N from the stover to the grain efficiently, particularly during the early stages of embryo development, avoiding embryo or ear abortion [53]. Maize genotypes possessing high stay green capacities have greater tolerance to post-silking environmental stresses [54], which include reduction in N uptake and prolonged leaf longevity [55] to sustain photosynthetic activity at a time of decline but high crop N demand [54]. These attributes make stay green characteristic an important component of genetic variation in NUE in maize [2]. The presence of genetic variation in NUE implies that

NUE could be assessed and improved genetically through selection [56], making it possible to breed new cultivars possessing better adaptation to nitrogen-deficient environments. Genetic variation for NUE is expressed differently at low and high-N-input. Therefore, genes for adaptation to N stress can only be observed under sufficiently low-N input [2,57]. The maximum genetic advance at low-N-input is better achieved when selection is conducted in low-N environments [53]. In low-N environments, genetic variance is usually reduced. For example, studies by Bertin and Gallais [51] under both low and high-N inputs demonstrated a reduction in genetic variance in NUE under low-N input. A high correlation between grain yield and kernel number was reported by the authors across varying nitrogen levels and a complete lack of  $G \times N$  interaction for plant traits at silking. These results indicated that grain yield is largely influenced by kernel numbers which was determined by nitrogen use. In addition to the use of the conventional breeding methods in the genetic enhancement of the early and extra-early maturing maize, molecular tools have been adopted as adjuncts to accelerate the genetic gains in the improvement of early and extra-early maize for low-N tolerance. The progress made in the use of molecular tools, as reviewed herein (see later) has focused on the assessment of genetic diversity and population structure, genome-wide association mapping (GWAS), and identification of candidate genes underlying traits involved in NUE.

### **Inter-Trait Relationships in Early and Extra-Early Maturing Maize Germplasm Under Low-Soil Nitrogen Environments**

Grain yield is a complex trait with low heritability under stress environments. As a result, improvement progress based on direct selection for yield alone is usually too slow and inefficient [58]. Under low-N conditions, selection for grain yield alongside secondary traits may help increase selection efficiency provided the secondary traits have adaptive value, relatively high heritability, significant genetic correlation with grain yield, and are easy to measure [58,59]. Therefore, it is crucial to identify secondary traits and estimate their values for use in index selection for improved grain yield in low-N environments.

Badu-Apraku et al. [60] studied 90 extra-early maturing maize inbred lines under low-N and reported large and significant genetic and phenotypic correlations between grain yield and stress-adaptive secondary traits excluding anthesis-silking interval and stalk lodging. In a similar study, Badu-Apraku et al. [61] using genotype  $\times$  trait (GT) biplot analysis, found that days to anthesis and silking, stay green characteristic, anthesis-silking interval, plant height, and ears per plant, as well as plant and ear aspects showed strong and significant correlation with yield. The authors concluded that anthesis-silking interval, ears per plant, and plant and ear aspects were the most reliable traits for indirect selection for improved yield potential among early maturing maize inbred lines under low-N environment. Based on these results, a base index involving

increased grain yield, reduced anthesis-silking interval, increased ears per plant, good stay green characteristic, and desirable plant and ear aspect scores, was formulated to characterize early and extra-early maturing maize germplasm for tolerance to low-N in WCA. Several studies have been conducted to confirm the appropriateness and to gain insight into the interrelationship among the traits used in the base index for selection for improved grain yield under low-N environments. For example, ear height, plant aspect, ear aspect, and stay green characteristic were identified by both path-coefficient and genotype main effect plus genotype  $\times$  environment interaction (GGE) biplot analyses as reliable traits for selecting for low-N tolerant extra-early maize inbred lines [60]. In the same study, the authors reported that a strong positive correlation existed among the following traits: stay green characteristic, plant, and ear aspects, and husk cover, suggesting that measuring just one of these traits will suffice without sacrificing important information on the genotypes. It is striking to note, however, that ears per plant and anthesis-silking interval, which have been used in the base index, were not among the reliable traits for selecting low-N tolerant genotypes [34]. In a study involving 56 extra-early open-pollinated maize cultivars developed during three breeding eras, plant aspect ( $P = -0.52$ ), stalk lodging ( $P = -0.42$ ), days to silking ( $P = -0.36$ ), plant height ( $P = -0.22$ ) and ear aspect ( $P = -0.18$ ) had direct effects on grain yield and accounted for 79.5% of its total variation [61]. These results, however, contradicted the findings of Talabi et al. [14] who analysed a maize panel of 250 early maturing full-sib progenies generated from North Carolina Design I. Using stepwise multiple regression analysis, the authors identified ear aspect, plant aspect, ears per plant, stay green characteristic, days to silking and stalk lodging as the most important secondary traits for yield improvement in low-N environments. Among these traits, ear aspect had the highest direct effect on grain yield ( $-0.419$ ), whereas only ears per plant (0.199) and ear height (0.160) had positive direct contributions to the observed 75% variation in grain yield [34]. Recently, Bhadmus et al. [35] conducted a genetic study on 96 early white quality protein maize (QPM) hybrids under low-N and identified plant and ear aspects as traits with significant direct effects on grain yield, accounting for about 73% of the total variation in grain yield. In their study, plant aspect had the highest direct effect ( $-0.58$ ) on grain yield while five traits (stay green characteristic, days to 50% silking, ear height, ears per plant, and plant height) contributed indirectly to grain yield through both plant and ear aspects. More recently, Badu-Apraku et al. [33] evaluated 54 early maturing maize hybrids developed during three breeding periods under low-N and reported a relative genetic gain of 2.91% per year and an average rate of increase in grain yield of 75.37 kg ha<sup>-1</sup> year<sup>-1</sup>. The reason adduced for the yield improvement was that the breeding strategy used under low-N resulted in delayed flowering; reduced anthesis-silking interval; improved husk cover, plant, and ear aspects; as well as improved stalk lodging [33].

From the foregoing, faster genetic progress in grain yield improvement of both early and extra-early maize germplasm under low-N is possible using plant and ear aspects, in combination with yield in a selection index. The traits were consistently associated with yield in several independent studies using both GGE biplot and path analyses. In addition to these traits, plant and ear heights were particularly important for extra-early maize and should be considered for inclusion in the IITA base index for characterizing extra-early maturity maize for low-N tolerance. Even though ears per plant, anthesis-silking interval and stay green characteristic were not consistently identified among the most reliable traits, their inclusion in index selection could further improve the precision with which low-N tolerant genotypes are identified. These seemingly important traits would have high value in maize improvement because they do not limit yield potential, have a higher heritability than yield alone, and are easy to determine because they are based on visual selection or counting.

### **GENETICS OF TOLERANCE TO LOW SOIL NITROGEN IN MAIZE**

The presence of genetic diversity for nitrogen use efficiency (NUE) in maize makes breeding for the trait an achievable objective [62,63]. The genes for NUE are well expressed under sufficiently low-N environments because under sufficient levels of nitrogen deficiency the influence of environment on gene expression is reduced whereas heritability of NUE and related secondary traits is increased [3]. Understanding the genetic mechanism of tolerance to low-N conditions is a pre-requisite for the success of a breeding program as it enables the design of the appropriate breeding strategies for the improvement of populations and hybrid development. Research on the genetics of low-N began several decades ago, but the literature is contradictory on the genetic basis of low-N tolerance in maize. One of the earliest reports was the research of Pollmer et al. [64] which found that both additive and non-additive gene effects contributed significantly to the uptake and translocation of nitrogen in maize genotypes selected from three different source populations. However, other earlier literature [8,28,65] have reported non-additive gene action to be more important, while Rizzi et al. [66], Bellow et al. [67] and Kling et al. [68] reported additive gene action to be more important. Under the IITA maize improvement program, several studies undertaken on the genetics of low-N using germplasms from different source populations have also yielded contradictory reports. Findings from studies conducted more recently suggest that additive gene action is more important in determining the inheritance of grain yield and other agronomic traits under low-N [7,10,35,57,63,69–74]. On the contrary Makumbi et al. [75], Badu-Apraku et al. [76], and Mafouasson et al. [77] reported that the inheritance of low-N tolerance is modulated by non-additive gene action. The inconsistencies in reports on the gene action for low-N tolerance could be attributed to the differences in the germplasm used for these studies.

This implies that the gene action for low-N tolerance is germplasm dependent, hence it is important to determine the gene action for newly developed inbred lines before utilizing them in hybrid programs to maximize heterosis. One of the recent studies on the mode of inheritance of low-N tolerance in the IITA early maturing inbred lines was conducted by Obeng-Bio et al. [7]. This research involved 24 early maturing provitamin A quality protein maize (PVA-QPM) inbred lines, which were evaluated under drought, low-N, and optimal growing conditions in Nigeria for two years. It was observed that additive genetic effects were greater than non-additive effects for grain yield and most agronomic traits under each and across the three environments. Two of the 24 inbred lines, TZEQI 82 and TZEIORQ 29 were identified to have significant and positive GCA-male and female effects for grain yield and stay green characteristic under low-N and across environments. These invaluable inbred lines could be used as either male or female parents for developing low-N tolerant hybrids. Furthermore, TZEIORQ 29 could be exploited for PVA favourable alleles in the development of superior hybrids to improve the nutritional security of consumers of high maize-dependent diets in SSA.

#### **Heterosis, Heterotic Grouping and Identification of Inbred and Single Cross Testers Under Low Soil Nitrogen Environments**

A vital component of hybrid maize breeding programme is the development of a set of inbred lines that maximize the manifestation of heterosis in hybrid combinations. The term heterosis was coined by Shull [78], to express the uncommon vigour of a hybrid or  $F_1$  generation resulting from the hybridization of two inbred lines of maize [79]. Heterosis can be defined as the amount by which the mean of any  $F_1$  family surpasses its better parent value (better parent heterosis) or mid-parent value (mid-parent heterosis) [80]. According to Ige et al. [81], mid-parent heterosis is not utilizable in practical plant breeding, because it does not offer the hybrid any advantage over the better parent. Two important theories to explain the phenomenon of heterosis are: (i) the dominance theory proposed by Keeble and Pellow [82] who suggested that increase in vigour after crossing resulted from the combination of various dominant alleles by each parent and (ii) the over-dominance theory as established by Shull [83] and East and Hays [84], who modified the dominance theory and suggested over-dominance hypothesis which states that heterosis is as the result of heterozygosis. Gardner et al. [85] and Moll et al. [86] were later able to show that dominance is the principal cause of heterosis.

The manifestation of heterosis depends on the genetic diversity of the two parents Moreno-Gonzales and Dudley [87]. Two parental varieties which exhibit a large amount of heterosis from their hybrids can be termed to be genetically more varied than two varieties that manifest little or no heterosis in their hybrids. It is reported in maize production that the range of heterosis contributes between 15 and 50% of the total grain yields [88]. A high amount of heterosis for grain yield, grain weight per ear, ear



weight, shelling percentage, moderate estimates for plant and ear height, ear circumference, number of kernels per ear row, number of kernel rows per ear, and grain weight were reported by Saleh et al. [89]. Meseka et al. [28] evaluated under low and high nitrogen conditions, 96 hybrids derived from 24 inbred lines using the North Carolina Design II. The authors observed average heterosis for grain yield of 129% under low nitrogen and 114% under high nitrogen. Nigussie et al. [90], in a study carried out to determine the combining ability and heterosis of eight elite maize genotypes indicated that the crossing of maize inbred lines developed from different source germplasm could result in better use of hybrid vigour. Ige et al. [81] evaluated ten open-pollinated varieties (OPVs) and their parental crosses under high-N and low-N conditions in Nigeria and found mid and better parent heterosis for grain yield of 29.14 and 19.81%, respectively. Under high-N, mid and better-parent heterosis for hybrids were 30.98% and 18.94%, respectively. Hybrid, DMR-LSR-W × TZSR-Y-I displayed the highest mid (97.70%) and high-parent (87.15%) heterosis for grain yield under high-N. However, the same hybrid expressed very low mid (9.26%) and high-parent (4.99%) heterosis under low-N environment, an indication that the hybrid was only suitable for cultivation in high-N environments. Ifie [91] evaluated 150 early single-cross hybrids developed from 30 inbred lines using the North Carolina Design II under low-N and recorded a mean mid and high parent heterosis of 156 and 112%, respectively for grain yield suggesting that the hybrids produced more ears and higher grain yield than the parents. Annor [92] also evaluated 205 hybrids developed by crossing 41 inbred lines with five inbred testers under three low-N environments in Nigeria for two years (2015 and 2016). The author reported that mid-parent heterosis for grain yield under low-N ranged from -9 to 241% with an average of 102% whereas the better parent heterosis ranged from -13 to 180% with an average of 75% (Table 3). The best 20 low-N tolerant hybrids displayed moderate to high positive mid- and better-parent heterosis for grain yield. According to the author, the hybrids will be priceless for production by the resource-poor maize farmers in WCA since the hybrids could produce high grain yields on soils with limited N. Moreover, negative mid- and better parent heterosis were estimated for days to silking and anthesis under low-N environments for most of the promising hybrids (Table 3), indicating that the hybrids matured earlier than their corresponding inbred parents in the different environments. However, positive heterosis values were recorded for ear and plant heights under low-N environments, an indication that the hybrids were taller than the parents.

**Table 3.** Estimates of heterosis for grain yield and other agronomic traits of early maturing maize hybrids (the best 20 low-N tolerant and worst 10 susceptible hybrids selected using the base index) under low-N environments.

HYBRID	YIELD		POLLEN		ASI		PLHT		PASP		EASP		EPP		STGR	
	MP	BP	MP	BP	MP	BP	MP	BP	MP	BP	MP	BP	MP	BP	MP	BP
TZEI 443 × ENT 13	163.69	139.55	-8.69	-6.56	-24.26	865.63	54.35	35.96	-13.51	-6.05	-7.88	-6.47	1.17	-4.99	48.31	106.85
TZEI 462 × TZEI 17	160.07	125.38	-6.48	-5.87	-77.33	285.42	30.83	18.91	71.75	84.16	15.46	36.87	5.38	4.69	1.62	16.76
ENT 8 × TZEI 10	184.37	174.96	-7.39	-6.65	-61.08	-8.54	44.39	39.74	-4.83	-2.67	-20.06	-14.67	21.36	19.57	43.79	54.24
ENT 8 × TZEI 17	149.34	130.89	-7.50	-5.61	-97.73	-87.50	40.90	39.18	24.74	66.06	18.32	52.88	3.17	-3.16	68.71	83.07
TZEI 455 × ENT 13	241.44	180.33	-10.15	-9.33	-61.99	-52.78	44.21	38.10	-22.21	-16.21	-0.28	1.62	-7.16	-7.69	19.90	41.05
TZEI 461 × TZEI 10	153.01	139.16	-6.37	-6.05	-93.86	16.67	38.02	38.00	15.73	31.31	-10.92	-4.55	16.00	10.95	34.90	37.04
TZEI 465 × ENT 13	124.93	92.99	-9.69	-8.30	-58.27	-0.48	32.95	30.55	5.67	17.57	4.56	0.57	-6.46	-11.82	20.95	28.95
TZEI 443 × TZEI 17	93.59	92.97	-8.56	-7.86	595.83	943.75	45.60	39.19	34.67	61.45	19.57	50.47	4.93	2.15	81.27	102.74
TZEI 16 × TZEI 23	124.92	108.92	-9.14	-3.44	-23.85	62.69	24.17	19.25	14.34	18.58	0.77	3.38	-2.99	-5.70	56.77	94.10
TZEI 462 × TZEI 10	176.76	165.90	-7.54	-7.02	-22.26	-17.55	27.61	20.97	29.33	54.52	-5.13	9.65	13.57	5.78	11.79	13.63
TZEI 516 × ENT 13	141.64	127.56	-9.55	-8.36	-58.09	-25.00	15.67	11.31	3.55	14.64	4.26	5.06	-10.37	-12.85	40.75	51.48
ENT 8 × TZEI 23	121.72	103.97	-7.26	-4.21	-50.00	-20.00	33.52	28.75	13.28	29.01	15.34	24.32	9.39	-1.47	57.26	67.27
TZEI 474 × TZEI 17	118.09	86.97	-7.70	-7.10	34.07	1039.58	29.14	20.81	32.42	71.51	28.65	64.07	9.63	5.51	32.27	54.79
TZEI 515 × TZEI 129	205.96	135.14	-11.03	-9.80	-81.00	-68.87	48.35	27.12	-13.33	-20.55	-15.32	-15.06	4.52	-9.00	47.65	56.88
ENT 17 × TZEI 10	193.32	158.92	-9.04	-7.36	45.36	356.85	20.41	14.26	-9.44	-4.64	-8.88	-7.10	15.85	13.29	-7.82	25.83
TZEI 494 × TZEI 10	232.19	151.37	-9.42	-8.03	-64.77	-59.91	40.60	32.94	2.71	10.50	-16.89	-14.04	27.59	19.47	75.33	133.00
TZEI 462 × ENT 13	156.43	142.56	-7.11	-6.29	-70.22	-63.01	20.32	19.05	11.17	35.15	2.06	-1.45	-3.36	-7.46	62.47	71.79
TZEI 24 × ENT 13	98.50	67.32	-10.29	-9.58	-66.49	-47.55	37.90	34.80	-9.84	-10.83	0.04	6.54	10.98	-6.43	66.44	75.99
TZEI 461 × TZEI 17	85.16	75.30	-5.98	-4.50	125.00	125.00	40.77	34.60	52.87	72.21	17.74	51.47	7.64	4.01	61.27	92.15
TZEI 430 × TZEI 10	124.59	116.15	-7.69	-4.51	-59.46	-29.72	46.10	39.17	13.92	19.62	-6.23	-14.14	12.31	7.58	3.39	14.05
TZEI 518 × TZEI 23	17.35	3.30	-6.13	-2.73	-4.70	34.28	15.01	14.60	50.48	58.83	24.18	47.59	0.08	-6.12	72.40	83.37
TZEI 428 × TZEI 129	107.45	75.06	-8.94	-7.00	-2.98	4.12	33.34	24.95	-1.26	-9.18	6.79	0.41	14.70	9.50	79.20	98.40
TZEI 432 × TZEI 10	24.80	15.50	-2.41	-1.76	-59.62	-48.70	27.22	25.81	17.75	19.98	13.89	6.42	-16.08	-22.09	11.69	41.83
TZEI 516 × TZEI 23	-8.71	-12.48	-6.55	-1.67	116.46	101.70	18.49	9.77	61.67	64.66	43.46	49.93	0.27	-3.21	35.40	38.84
TZEI 16 × TZEI 17	29.11	20.73	-6.23	-5.55	19.92	4277.08	15.37	5.80	69.74	103.49	64.51	89.47	-19.64	-20.94	113.86	126.59
TZEI 433 × TZEI 23	19.10	-9.16	-5.22	-1.52	-5.68	17.90	11.11	2.59	36.56	67.53	27.33	47.68	-11.12	-12.54	81.99	103.83
TZEI 432 × TZEI 17	55.39	30.31	-4.51	-2.69	139.94	6858.33	33.99	29.53	42.77	89.21	40.57	80.06	-27.18	-27.40	33.36	104.30
TZEI 182 × TZEI 10	0.63	-6.33	-5.58	-4.52	-36.32	-18.24	13.41	8.49	36.37	43.82	23.18	34.58	0.03	-4.23	77.99	108.29
TZEI 439 × TZEI 23	2.76	-7.78	-4.41	0.84	-1.34	39.02	16.26	13.14	62.69	64.20	32.85	39.38	-16.12	-20.37	70.83	73.59
TZEI 522 × TZEI 23	-4.62	-12.72	-5.81	-3.61	-14.57	123.30	22.43	21.55	62.11	65.22	51.17	54.40	-15.25	-15.44	92.52	128.89
Average	102.08	75.35	-7.71	-5.96	-12.84	382.57	27.31	21.22	21.57	35.32	13.80	26.94	0.66	-6.02	60.53	81.56

Note: MP = mid-parent heterosis; BP = better-parent heterosis; YIELD = grain yield (kg ha<sup>-1</sup>); Pollen = days to 50% anthesis; ASI = anthesis-silking interval; PLHT = plant height (cm); PASP = plant aspect; EASP = ear aspect; EPP = ears per plant; STGR = stay-green characteristic.

To exploit maximum heterosis in maize, inbred lines are classified into different heterotic groups before crosses are made in breeding programmes. Many investigators have indicated that the combination of lines from different heterotic groups results in hybrids with better chances of genetic expression of the target character [69,93–98]. A heterotic group, according to Melchinger and Gumber [99] may be defined as a group of related or unrelated cultivars from the same or different populations which exhibit(s) similar combining ability and heterotic response when crossed with cultivars from genetically distinct germplasm groups. On the other hand, heterotic pattern refers to two heterotic groups which display high hybrid performance and heterosis in their crosses. According to Ricci et al. [100] and Annor et al. [94], assigning inbred lines to heterotic groups would prevent the development and evaluation of crosses that should be discarded, allowing maximum heterosis to be exploited by crossing inbred lines belonging to different heterotic groups. The establishment of heterotic patterns among varieties is crucial in selecting inbred lines as parental seed stocks in hybrid production [101]. Mating designs that can be employed to classify genotypes into heterotic groups include diallel [10,98], the line  $\times$  tester [94,95], and the North Carolina Design II [61]. Numerous methods have been proposed for heterotic grouping of inbred lines and are presently used widely across the world. The specific combining ability (SCA) effects of grain yield is the traditional method used to classify genotypes into heterotic groups [102]. The heterotic grouping based on the SCA of grain yield method has been utilized by several researchers [95,103,104]. Nonetheless, the SCA effects of grain yield have often been influenced by the interaction between environments and genotypes, resulting in the classification of the same genotypes into different heterotic groups in different research [69]. Owing to the inconsistency of the SCA heterotic grouping method, the use of both SCA and general combining ability (GCA) effects of grain yield was proposed by Fan et al. [97]. The method, designated as heterotic group's specific and general combining ability effects of grain yield (HSGCA), was found to be more efficient for allocating inbred lines into heterotic groups. However, both methods (HSGCA or SCA) are based on only grain yield which poses a serious challenge [69].

Grain yield is a quantitatively inherited trait which is controlled by many genes. It also has low heritability, especially under stress conditions, and affected by other traits. Badu-Apraku et al. [69] therefore proposed a heterotic grouping method based on GCA of multiple traits (HGCAMT). The proposed method is based on several traits of inbred lines with significant GCA effects. The HGCAMT is very important, particularly in the NCD II where crosses are not made among all inbred lines involved. It is appropriate to allocate genotypes to heterotic groups based on multiple traits, principally in a situation where hybrids or varieties are being developed for resistance or tolerance to several stresses.

Over the years, efforts by various investigators to identify the most efficient method for allocating maize genotypes into heterotic groups have

discovered contradictory results. For instance, Amegbor et al. [94] found the SCA method to be less efficient than the HSGCA method. The HSGCA, molecular marker, and SCA classification methods were compared by Fan et al. [97] and Akinwale et al. [105], and the HSGCA method was found to be the most efficient. Furthermore, the HSGCA method had a higher breeding efficiency than the SNP-based grouping method when they were compared by Annor et al. [94]. Similarly, the HSGCA method was found to be the most efficient compared to the SNP marker-based, HGCAMT, and the SCA methods by Badu-Apraku et al. [10]. In contrast, Badu-Apraku et al. [71] found the molecular marker method to be the most efficient when compared to the SCA, HSGCA, and HGCAMT. Also, the HGCAMT was found to be the most efficient compared to the HSGCA and SNP-based molecular methods in a study conducted by Badu-Apraku et al. [76]. In addition, Badu-Apraku et al. [69] compared to the HSGCA, HGCAMT, SCA, and SSR-based molecular marker methods and found that the HGCAMT was more effective, followed by the HSGCA method. The inconsistencies found in the different studies could be attributed to the variances in the genetic materials used.

An important prerequisite of any successful low-N tolerant maize improvement programme is the availability of efficient testers which could differentiate and classify maize inbred lines into proper heterotic groups. The tester should be appropriate for the development of outstanding synthetic varieties and hybrids [35,69,94,106]. According to Pswarayi and Vivek [107], an inbred line could be selected as a potential tester based on the following criteria: the inbred line should (i) be classified into a particular heterotic group; (ii) have a high *per se* grain yield performance, and (iii) have a positive and significant GCA effect across the test environments. Based on these criteria, several inbred lines have been identified by several authors [10,35,69,71,76,107]. On the other hand, identification of the single-cross hybrids as a tester is based on (i) display of good GCA effects of the inbred lines constituting the single cross, (ii) grouping of the inbred lines constituting the single cross to the same heterotic group, and (iii) a good yielding ability of the potential single-cross tester to qualify its use as a seed parent in successful three-way and double-cross hybrids for high seed production. Based on these criteria, Pswarayi and Vivek [107], Badu-Apraku et al. [69], Annor [92], and Bhadmus et al. [35] have identified low-N tolerant single-cross testers for use by maize breeders in SSA.

### **Genetic Gains from Selection for Tolerance to Low Soil Nitrogen in Maize**

Genetic gain, one of the important concepts in conventional quantitative genetics and breeding can be explained as the level of increase in performance that is achieved annually through artificial selection. This is also tied to the genetic diversity of source population(s) as it affects the potential genetic gain through selection. Genetic gains in traits are typically tracked between a base or source population (unselected population) and progeny generated from parents selected from the source population. Thus,

the improvement made in the progeny for a trait is usually estimated using the “breeder’s equation”;  $R = h^2 \times S$ , where  $R$  is response to selection,  $h^2$  is heritability, and  $S$  is selection differential (the difference between the mean of the source population and the mean of the parental population). Response to selection ( $R$ ) generally represents the absolute gain, and therefore, gains per unit time ( $\Delta g$ ) is quantified as  $\Delta g = R/L$ , where  $L$  is the unit time involved in the improvement of the trait. The concept of genetic gain is also applied in instances where the performance of old and new generation hybrids or cultivars are compared to monitor the level of progress made from one breeding period to the other. In this case, regression analysis using linear or quadratic relationship models is performed between a trait and the period of improvement to estimate the slope (b value), and this value divided by the intercept represents the absolute gain in that trait. The absolute gain divided by the years of improvement per period represents the relative gain per that period [3].

### **Genetic Gains from Selection for Low-N Tolerance in the IITA Maize Improvement Programme**

Breeding effort towards the development of low soil nitrogen (low-N) tolerant early and extra-early maize genotypes in the IITA Maize Improvement Program (IITA-MIP) commenced more than two decades ago in collaboration with the National Agricultural Research Systems (NARS) in SSA. During this period, early and extra-early maturing maize germplasm with good NUE were identified following several years of extensive testing in SSA and have been used as parents to develop adapted source populations for improvement using recurrent selection procedures. Several promising early-maturing varieties with high grain yielding ability under low-N have been identified and commercialized mainly through NARS in SSA. Outstanding newer cultivars are still being developed in the IITA-MIP and NARS under low-N conditions to replace older varieties that were once superior. Thus, the breeding pipeline for the development of low-N tolerant early and extra-early maize cultivars at the IITA-MIP and partners consistently remain very functional. To this end, it has been very imperative to track and quantify the performance of cultivars over different generations to provide information on the level of improvement made for key traits under low-N conditions. This strategy helps to guide breeding processes for the development of superior low-N tolerant early and extra-early maize varieties for SSA. Achieving this objective has always required genetic gain studies for grain yield and other important traits under low-N conditions.

It suffices to note that drought, low-N, and infestation by *Striga hermonthica* Del. (Benth) which constitute the three main constraints to maize production and productivity in SSA, practically act and interact with devastating synergistic effects. Studies conducted in WCA on the combined effects of the three stress factors indicated 44 to 53% grain yield reduction by drought stress, 42 to 65% by *Striga* infestation, and 40% by low-N [108,109]. In this review, emphasis is placed on the genetic gain under low-N conditions

because investigations under *Striga*-infested and drought environments have received much attention relative to that under low-N environments. However, in the agro-ecologies of SSA, limited N availability is equally a common phenomenon [4,30]. Limited N levels in soils of SSA and the associated challenges faced by farmers to resolve the problem continue to reduce the grain yield potential of cultivars in the sub-region. For example, Obeng-Bio et al. [110] estimated wider range (10–71%) of annual yield reduction due to low-N.

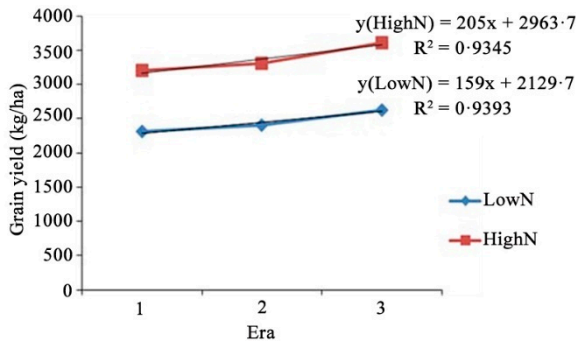
Unlike under low-N conditions, this knowledge gap is gradually becoming narrower for studies under *Striga* infestation [69,111,112] and drought conditions [10,61,69,112–114]. However, some genetic gain studies under low-N environments were conducted close to two decades ago to provide information on absolute gains that could be realized from the improvement in grain yield. For example, Omoigui et al. [115] adopted the full-sib family selection breeding strategy to improve tropical maize populations for low-N tolerance. Genetic gains of 2.3 and 1.9 per cycle for grain yield under low- and high-N respectively, were estimated following three cycles of full-sib recurrent selection for low-N tolerance. The authors also observed increased stay green characteristic and kernel weight with corresponding gains of 17.7 and 4.7 per cycle, respectively. The magnitude of change in the average grain yield and other important agronomic traits after three cycles of selection indicated changes in gene frequencies due to the significant improvement made in the tropical maize populations used.

In the past decade, genetic gain studies conducted for the early and extra-early maturing maize cultivars within the IITA-MIP have revealed interesting results to aid the understanding of cultivar performance from one breeding period to the other. For instance, Badu-Apraku et al. [111] evaluated a set of 50 early maturing cultivars developed during the three breeding periods to test the hypothesis that tolerance to low-N had been improved while selecting for drought tolerance and *Striga* resistance and the authors also estimated genetic gain in grain yield under low-N conditions for the cultivars. Results showed that selection for *Striga* resistance and drought tolerance in the early maturing maize populations also enhanced low-N tolerance. Under low-N conditions, grain yield improved from 2280 kg ha<sup>-1</sup> during the first period to 2610 kg ha<sup>-1</sup> during the third period with an absolute increase of 165 kg ha<sup>-1</sup> per period, and  $r^2$  of 0.94. Under high-N conditions, grain yield increased from 3200 to 3650 kg ha<sup>-1</sup> with an absolute increase of 225 kg ha<sup>-1</sup> per period, and  $r^2$  of 0.93 (Figure 1). Relative gain per period (that is, gain in grain yield in a period per unit yield in the previous period) was the same (30 kg ha<sup>-1</sup>) for both the high-N and low-N rates with  $r^2$  values of 0.99 and 0.94, respectively. Regression analysis of grain yield under the N environments showed a positive predictive relationship between one level and the other, with performance under low-N predicting performance under high-N better than *vice versa* (Figures 2a and 2b). Thus, substantial progress was made as grain yield improvement was higher in later than earlier breeding periods. In another study, Badu-Apraku et al. [112]

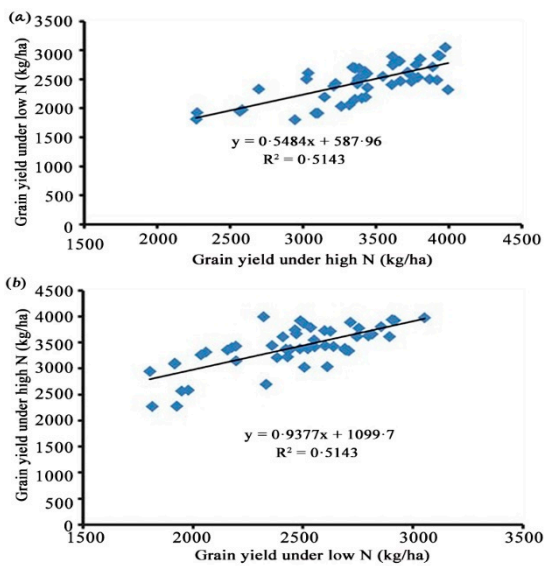
evaluated 56 extra-early open-pollinated maize cultivars developed during three breeding eras, 1995–2000, 2001–2006, and 2007–2012, under low- and high-N conditions at two locations in Nigeria to investigate the genetic gains in grain yield. The third generation of the extra-early maize cultivars (era 3) showed a significant increase in grain yield compared to those developed during the initial two eras under low- and high-N conditions (Table 4). That is, the low-N experiments revealed grain yield increased from 2.65 Mg ha<sup>-1</sup> in the first era to 3.27 Mg ha<sup>-1</sup> in the third era. Similarly, the high-N experiments showed yield increase from 3.41 Mg ha<sup>-1</sup> in the first era to 4.40 Mg ha<sup>-1</sup> in the third era. Grain yield reduction under low-N compared with that under high-N was 24%.

**Table 4.** Grain yield and other agronomic traits of extra-early maize cultivars of three breeding eras evaluated under low and high Nitrogen conditions at four locations in Nigeria between 2013 and 2014 growing seasons.

Trait	Era	No. of cultivars	Low-N	High-N
Grain yield, t ha <sup>-1</sup>	1995–2000	14	2.65 ± 0.05	3.41 ± 0.07
	2001–2006	17	3.05 ± 0.05	3.93 ± 0.06
	2007–2012	25	3.27 ± 0.04	4.40 ± 0.05
Days to anthesis	1995–2000	14	50 ± 0.18	49 ± 0.17
	2001–2006	17	52 ± 0.16	51 ± 0.15
	2007–2012	25	52 ± 0.13	51 ± 0.13
Days to silking	1995–2000	14	52 ± 0.18	50 ± 0.19
	2001–2006	17	53 ± 0.16	52 ± 0.17
	2007–2012	25	53 ± 0.13	52 ± 0.14
Anthesis–silking interval	1995–2000	14	2 ± 0.10	2 ± 0.09
	2001–2006	17	2 ± 0.09	1 ± 0.08
	2007–2012	25	2 ± 0.08	1 ± 0.07
Plant height, cm	1995–2000	14	163 ± 0.95	182 ± 1.26
	2001–2006	17	170 ± 0.85	190 ± 1.12
	2007–2012	25	170 ± 0.71	191 ± 0.94
Ear height, cm	1995–2000	14	79 ± 0.87	90 ± 0.98
	2001–2006	17	82 ± 0.77	97 ± 0.88
	2007–2012	25	82 ± 0.65	97 ± 0.74
Root lodging (%)	1995–2000	14	11.3 ± 0.59	10.6 ± 0.60
	2001–2006	17	10.7 ± 0.53	10.1 ± 0.54
	2007–2012	25	10.1 ± 0.44	8.8 ± 0.45
Stalk lodging (%)	1995–2000	14	17.5 ± 0.81	28.8 ± 1.05
	2001–2006	17	15.5 ± 0.72	26.5 ± 0.94
	2007–2012	25	11.7 ± 0.61	23.3 ± 0.79
Husk cover	1995–2000	14	2.4 ± 0.03	2.3 ± 0.03
	2001–2006	17	2.3 ± 0.03	2.2 ± 0.03
	2007–2012	25	2.3 ± 0.02	2.2 ± 0.02
Plant aspect	1995–2000	14	3.2 ± 0.03	3.1 ± 0.03
	2001–2006	17	3.0 ± 0.03	2.9 ± 0.03
	2007–2012	25	2.9 ± 0.02	2.7 ± 0.02
Stay green characteristic	1995–2000	14	4.6 ± 0.14	–
	2001–2006	17	4.3 ± 0.13	–
	2007–2012	25	4.0 ± 0.11	–
Ear aspect	1995–2000	14	3.3 ± 0.10	3.0 ± 0.03
	2001–2006	17	2.8 ± 0.09	2.8 ± 0.03
	2007–2012	25	2.7 ± 0.07	2.7 ± 0.02
Ear rot	1995–2000	14	2.5 ± 0.13	3.3 ± 0.15
	2001–2006	17	2.1 ± 0.11	2.7 ± 0.13
	2007–2012	25	2.3 ± 0.10	2.9 ± 0.11
Ears per plant	1995–2000	14	0.9 ± 0.001	0.9 ± 0.01
	2001–2006	17	0.9 ± 0.001	0.9 ± 0.01
	2007–2012	25	0.9 ± 0.001	0.9 ± 0.01



**Figure 1.** Relationship among grain yield of early maturing maize cultivars (kg ha<sup>-1</sup>) for three breeding eras evaluated under low- and high-nitrogen environments at Ile-Ife and Mokwa, Nigeria, 2010 and 2011.

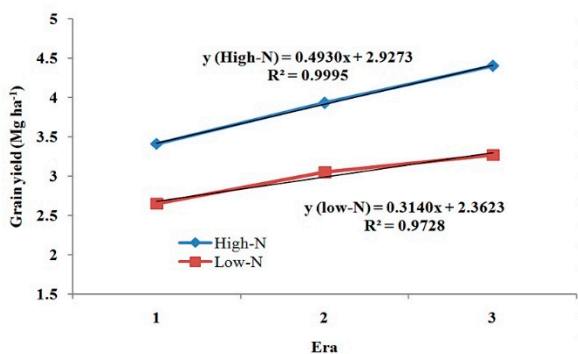


**Figure 2.** Regression of (a) grain yield of low-nitrogen (N) environments on grain yield of high-N environments and (b) grain yield of high-N environments on grain yield of low-N environments.

Additionally, the results revealed genetic gains in grain yield of 0.3140 Mg ha<sup>-1</sup> (13.29%) and 0.4930 Mg ha<sup>-1</sup> (16.84%) per era under low- and high-N, respectively (Figure 3). The annual genetic gain in grain yield was 0.054 Mg ha<sup>-1</sup> (2.14%) under low-N and 0.081 Mg ha<sup>-1</sup> (2.56%) under high-N conditions (Table 5). The study revealed significant progress made in breeding for high grain yield and low-N tolerance among the 56 extra-early open-pollinated maize cultivars representing three breeding eras from 1995 to 2012. More recently, 54 early maturing hybrids developed during three breeding periods were evaluated under low-N (30 kg ha<sup>-1</sup>) and high-N (120 kg ha<sup>-1</sup>) across two locations, Ile-Ife and Mokwa, in Nigeria, from 2017 to 2019 [62]. Amongst the objectives of the study was the determination of genetic gains in grain yield of the early-maturing hybrids. The results revealed a significant ( $p < 0.05$ ) gain in grain yield in the third period (period 3) of the early maize hybrids relative to those in the first and second periods under low- and high-N conditions (Table 5). In low-N environments, grain yield improved from 2784



kg ha<sup>-1</sup> in the first period to 2933 and 3197 kg ha<sup>-1</sup> in the second and third periods, respectively. Likewise, under high-N, grain yield increased from 4294 kg ha<sup>-1</sup> in period 1 to 4330 kg ha<sup>-1</sup> in period 2 and then to 4614 kg ha<sup>-1</sup> in period 3, although grain yield in period 2 was not significantly different from that of period 1 (Table 6). The results showed annual genetic gains in grain yield of 75 kg ha<sup>-1</sup> year<sup>-1</sup> with a relative gain of 2.91% under low-N conditions, and 55 kg ha<sup>-1</sup> year<sup>-1</sup> with a relative gain of 1.33% under high-N conditions (Table 6). Generally, the gains in grain yield observed from period 1 to 3 under both low- and high-N conditions was influenced by reduced ASI, and improvement in stalk lodging, husk cover, stay-green characteristic, as well as plant and ear aspects. Also, significant positive absolute gains (b values) were recorded for grain yield, plant height, and EPP. On the contrary, significant negative b values were observed for plant and ear aspects in both low- and high-N environments and ASI in low-N environments (Table 7).



**Figure 3.** Relationship among grain yield of extra-early maturing maize cultivars (Mg ha<sup>-1</sup>) for three breeding eras evaluated under low- and high N environments at Ile-Ife and Mokwa, Nigeria, 2013 and 2014.

**Table 5.** Relative genetic gain, coefficient of determination (R<sup>2</sup>), slope (a) and regression coefficients (b) of grain yield and other agronomic traits of 56 extra-early maize cultivars of three breeding eras evaluated under low-N and high-N conditions at four locations in Nigeria between 2013 and 2014 growing seasons.

Trait	Relative gain (%) per year)		R <sup>2</sup>		a		b	
	Low-N	High-N	Low-N	High-N	Low-N	High-N	Low-N	High-N
Grain yield (t ha <sup>-1</sup> )	2.14	2.56	0.513	0.587	2.52	3.16	0.054**	0.081**
Days to anthesis	0.14	0.21	0.078	0.185	50.60	49.55	0.069	0.105
Days to silking	0.08	0.08	0.032	0.118	52.84	51.10	0.040	0.087
Anthesis-silking interval	-1.11	-1.68	0.363	0.189	2.25	1.79	-0.025*	-0.030
Plant height (cm)	0.38	0.38	0.399	0.337	162.16	181.84	0.610*	0.682*
Ear height (cm)	0.33	0.30	0.117	0.157	78.77	92.72	0.257	0.280
Root lodging (%)	-0.68	-0.99	0.056	0.098	10.76	10.48	-0.073	-0.104
Stalk lodging (%)	-2.65	-1.86	0.323	0.332	18.67	31.89	-0.494*	-0.592*
Husk cover	-0.64	-0.59	0.284	0.473	2.51	2.38	-0.016*	-0.014**
Plant aspect	-0.85	-0.87	0.510	0.663	3.29	3.12	0.028**	-0.027**
Ear aspect	-1.11	-0.97	0.404	0.480	3.25	3.09	-0.036*	-0.030**
Ear rot	-1.76	-2.11	0.211	0.350	2.73	3.80	-0.048	-0.080*
Ears/plant	-0.22	0.11	0.197	0.125	0.91	0.89	-0.002	0.001
Stay green characteristic	-1.51	-	0.316	-	4.91	-	-0.074*	-

Note: \*, \*\* significant at 0.05 and 0.01 probability levels.

**Table 6.** Grain yield and other agronomic traits of eighteen early-maturing maize hybrids of three breeding periods under low-N in four environments and high-N in five environments in Nigeria from 2017 to 2019.

Trait	Periods	Low-N condition	High-N condition
Grain yield (kg ha <sup>-1</sup> )	2008–2010	2784.03 ± 75.11	4294.33 ± 111.79
	2011–2013	2932.54 ± 91.12	4330.10 ± 99.38
	2014–2016	3196.79 ± 66.59	4614.44 ± 116.52
Days to anthesis	2008–2010	53.19 ± 0.17	51.28 ± 0.21
	2011–2013	53.06 ± 0.23	51.29 ± 0.27
	2014–2016	53.09 ± 0.28	51.77 ± 0.35
Days to silking	2008–2010	53.99 ± 0.16	52.42 ± 0.21
	2011–2013	53.96 ± 0.23	52.52 ± 0.30
	2014–2016	53.78 ± 0.32	52.65 ± 0.37
Anthesis-silking interval	2008–2010	0.85 ± 0.07	1.33 ± 0.07
	2011–2013	0.91 ± 0.78	1.23 ± 0.07
	2014–2016	0.78 ± 0.06	1.05 ± 0.09
Plant height (cm)	2008–2010	157.03 ± 1.95	161.76 ± 2.42
	2011–2013	165.42 ± 2.55	170.53 ± 3.20
	2014–2016	164.18 ± 2.20	171.49 ± 2.17
Ear height (cm)	2008–2010	70.86 ± 1.46	73.96 ± 1.60
	2011–2013	74.58 ± 1.60	79.29 ± 1.91
	2014–2016	71.94 ± 1.11	76.64 ± 1.39
Root lodging %	2008–2010	0.90 ± 0.17	4.75 ± 0.89
	2011–2013	0.70 ± 0.16	5.58 ± 0.77
	2014–2016	0.99 ± 0.22	4.98 ± 0.68
Stalk lodging %	2008–2010	6.17 ± 0.34	2.89 ± 0.32
	2011–2013	6.77 ± 0.41	3.10 ± 0.40
	2014–2016	5.14 ± 0.35	2.55 ± 0.33
Husk cover	2008–2010	3.11 ± 0.06	3.41 ± 0.05
	2011–2013	3.20 ± 0.08	3.41 ± 0.06
	2014–2016	2.94 ± 0.05	3.25 ± 0.07
Plant aspect	2008–2010	4.76 ± 0.05	4.47 ± 0.06
	2011–2013	4.61 ± 0.08	4.35 ± 0.06
	2014–2016	4.39 ± 0.06	4.23 ± 0.06
Ear aspect	2008–2010	4.65 ± 0.08	3.92 ± 0.09
	2011–2013	4.34 ± 0.10	3.76 ± 0.07
	2014–2016	4.42 ± 0.07	3.69 ± 0.08
Ear rot	2008–2010	8.48 ± 0.51	6.04 ± 0.33
	2011–2013	8.51 ± 0.51	5.64 ± 0.42
	2014–2016	8.15 ± 0.58	5.81 ± 0.34
Ears per plant	2008–2010	0.80 ± 0.01	0.82 ± 0.01
	2011–2013	0.82 ± 0.01	0.86 ± 0.01
	2014–2016	0.84 ± 0.01	0.86 ± 0.01
Stay-green characteristic	2008–2010	3.31 ± 0.09	–
	2011–2013	3.22 ± 0.08	–
	2014–2016	3.14 ± 0.09	–

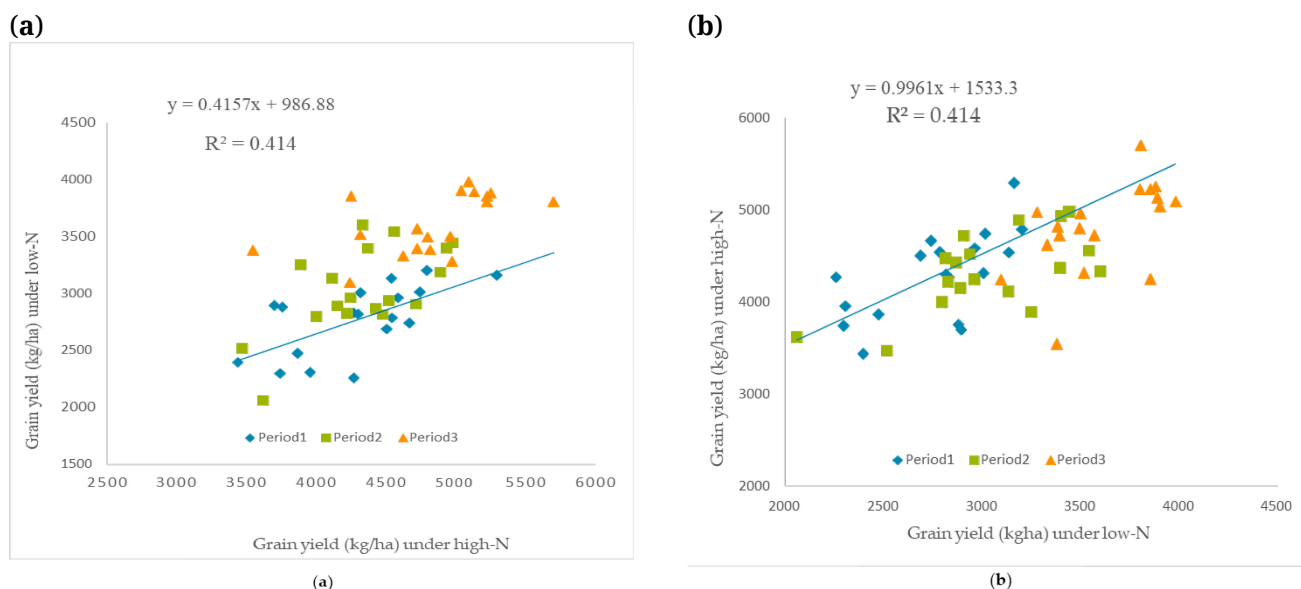
**Table 7.** Relative genetic gain, coefficient of determination ( $R^2$ ), slope (a) and regression coefficients (b) of grain yield and other agronomic traits of early-maturing maize hybrids during three breeding periods under low-N in four environments and high-N in five environments in Nigeria from 2017 to 2019.

Trait	Relative Gain			
	(% per Year)	$R^2$	a	b
Low-N condition				
Grain yield (kg/ha)	2.91	0.26	2588.7	75.37**
Days to anthesis	0.02	0.01	53.06	0.01ns
Days to silking	-0.02	0.00	53.95	-0.01ns
Anthesis-silking interval	-1.88	0.03	0.94	-0.02ns
Plant height (cm)	1.07	0.17	153.89	1.64**
Ear height (cm)	0.57	0.03	70.42	0.40ns
Root lodging (%)	3.12	0.01	0.75	0.02ns
Stalk lodging (%)	0.50	0.00	5.88	0.03ns
Husk cover	-0.33	0.01	3.14	-0.01ns
Plant aspect	-1.07	0.18	4.85	-0.05**
Ear aspect	-1.31	0.17	4.79	-0.06**
Ear rot	-0.80	0.01	8.74	-0.07ns
Stay-green characteristic	-0.89	0.04	3.38	-0.03ns
Ears/plant	1.08	0.27	0.78	0.01**
High- N condition				
Grain yield (kg/ha)	1.33	0.08	4133.3	55.12*
Days to anthesis	0.19	0.04	50.95	0.10ns
Days to silking	0.12	0.02	52.21	0.06ns
Anthesis-silking interval	-3.15	0.11	1.43	-0.05*
Plant height (cm)	1.11	0.14	159	1.76**
Ear height (cm)	0.84	0.05	73.51	0.62ns
Root lodging (%)	1.87	0.00	4.66	0.09ns
Stalk lodging (%)	-0.92	0.00	2.99	-0.03ns
Husk cover	-0.68	0.05	3.47	-0.02ns
Plant aspect	-0.95	0.15	4.57	-0.04**
Ear aspect	-0.99	0.08	3.99	-0.04*
Ear rot	-1.16	0.01	6.20	-0.07ns
Ears/plant	0.81	0.13	0.82	0.01**

Note: \*, \*\* significant at 0.05 and 0.01 probability levels, respectively; ns = non-significant.

Regression analysis of grain yield of the early maize hybrids under low-N on yield under high-N and *vice versa* (Figures 4a and 4b) undoubtedly distinguished period 3 hybrids as superior performers from those of the first and second periods. The results, therefore, affirmed the substantial genetic gains made in the improvement of the early-maturing hybrids over the periods of improvement in grain yield and other important agronomic traits. Sixty extra-early maturing maize hybrids developed during three breeding periods; 2008 to 2010, 2011 to 2013, and 2014 to 2016 were evaluated under low-N and high-N environments from 2017 to 2019. The study aimed at assessing the gain in grain yield of the hybrids and

identifying promising and stable hybrids across low- and high-N environments. Annual increase in yield of 64 kg ha<sup>-1</sup> year<sup>-1</sup> (2.59%) and 102 kg ha<sup>-1</sup> year<sup>-1</sup> (3.0%) was obtained under stress and non-stress environments, respectively. In a study to examine the yield gains in extra-early maize hybrids of three breeding periods under multiple environments, sixty extra-early maize hybrids developed during three breeding periods (2008–2010, 2011–2013, and 2014–2016) were evaluated under artificial *Striga* infestation at Abuja and Mokwa, low- and high-N at Ile-Ife and Mokwa, and optimal environments at Ikenne, Mokwa, and Abuja during the 2016 and 2017 growing seasons. The hybrids were also evaluated under optimal environments in Zaria and Bagauda in 2016. Results revealed that grain yield ranged from 1487 kg ha<sup>-1</sup> for (TZEEI 95 × TZEEI 58) × (TZEEI 82 × TZEEI 79) to 3009 kg ha<sup>-1</sup> for (TZEEI 29 × TZEEI 21) × TZEEI 58 across multiple stress environments. The annual genetic gain in grain yield was 43 kg ha<sup>-1</sup> (2.08 %) across multiple stress and 110 kg ha<sup>-1</sup> (3.15 %) across non-stress environments.



**Figure 4.** Comparative performance of early-maturing maize hybrids of the three breeding periods in low and high-N environments. Regression of (a) grain yield of low-N on grain yield of high-N and (b) grain yield of high-N on grain yield of low-N conditions.

### Molecular Approaches to Breeding Early and Extra-Early Maize for Tolerance to Low Soil Nitrogen at IITA

Understanding the molecular basis of how maize respond to changes in N availability would facilitate the development of strategies that are more effective in improving low-N tolerance and enhancing maize production in WCA. At IITA, molecular approaches used for the enhancement of low N tolerance in early and extra-early maize germplasm have focused on the assessment of genetic diversity and population structure, as well as genome-wide association mapping to uncover genomic regions and/or candidate genes underlying traits involved in low-N tolerance.

*Assessment of genetic diversity and population structure of early and extra-early maturing low soil nitrogen tolerant maize germplasm at IITA*

Information on genetic diversity is critical for efficient exploitation of inbred lines for population improvement and hybrid development for adaptation to multiple environmental conditions. Many studies have reported considerable genetic variability for tolerance to low-N in the IITA early and extra-early maturing maize germplasm, which could be harnessed for the development of new low-N tolerant cultivars [70,71,110,116,117]. Using 8171 single nucleotide polymorphism (SNP)-based DArTseq markers, Obeng-bio et al. [110] found that the genetic distance (GD) among 70 early maturing provitamin A (PVA) and quality protein (QPM) inbred lines ranged between 0.042 and 0.500. In their study, cluster analysis via principal coordinate and population structure analyses both classified the inbred lines into five distinct groups consistent with their pedigrees and performance under low-N conditions. Recently, Abu et al. [116] conducted a genetic diversity study using 110 extra-early yellow, orange, and white endosperm QPM inbred lines. They observed that the GD between the inbred lines varied from 0.019 to 0.607 based on 1691 informative SNP markers. The unweighted pair group method with arithmetic mean (UPGMA) and the admixture model-based population structure methods both classified the inbred lines into five clusters. The groups were consistent with the pedigree, and endosperm color but not with the low N tolerance levels of the inbred lines. Bhadmus et al. [117] found significant genetic diversity among 140 white endosperm QPM inbred lines derived from low-N tolerant populations. The study reported minor allele frequency (MAF) of 0.01–0.05 and the polymorphic information content (PIC) of 0.09–0.38 using 7599 informative SNP-based DArTseq markers. The significant genetic diversity observed in these studies indicated the presence of unique and beneficial alleles for introgression into populations to improve the tolerance to low-N. Furthermore, molecular marker-based diversity studies have been used to classify inbred lines into heterotic groups to increase their usefulness in the low-N breeding programmes. Using the genetic distances from 60 simple-sequence repeats (SSR) markers, Badu-Apraku et al. [69] grouped 20 extra-early inbred lines into three heterotic groups which was consistent with heterotic grouping produced by the general combining ability (GCA) effects of multiple traits (HGCAMT) procedure. A similar result was reported by Badu-Apraku et al. [71] in a study of the genetic diversity of 14 early maturing yellow endosperm inbred lines using 1451 SNPs. They also reported two heterotic groups for both genetic distance-based and the HGCAMT methods. The relationship between the SNP-based genetic distance and heterosis of hybrids remains undefined even though a significant correlation between molecular marker-based genetic distance and heterosis has been established [118]. This is due to the low predictivity of heterosis from genetic distances, particularly for inbred lines that exhibit wide genetic distance. However, Abu et al. [72] found a

significant correlation between SNP-based genetic distance and hybrid performance under low-N among 24 extra-early QPM inbred lines. In their study, inbred lines selected from two low-N tolerant populations (yellow QPM and orange QPM/PVA) and genotyped using 2500 SNP markers, clustered into four populations based on pedigree and endosperm colour and exhibited pairwise genetic distances between 0.036 and 0.602. The results revealed that the maximum heterosis and hybrid performance were in hybrids developed from parents TZEEIORQ 9A and TZEEQI 397, which had the highest genetic distance (0.602), while the worst performing hybrids were from parents TZEEQI 396 and TZEEQI 399 which had the lowest genetic distance (0.036).

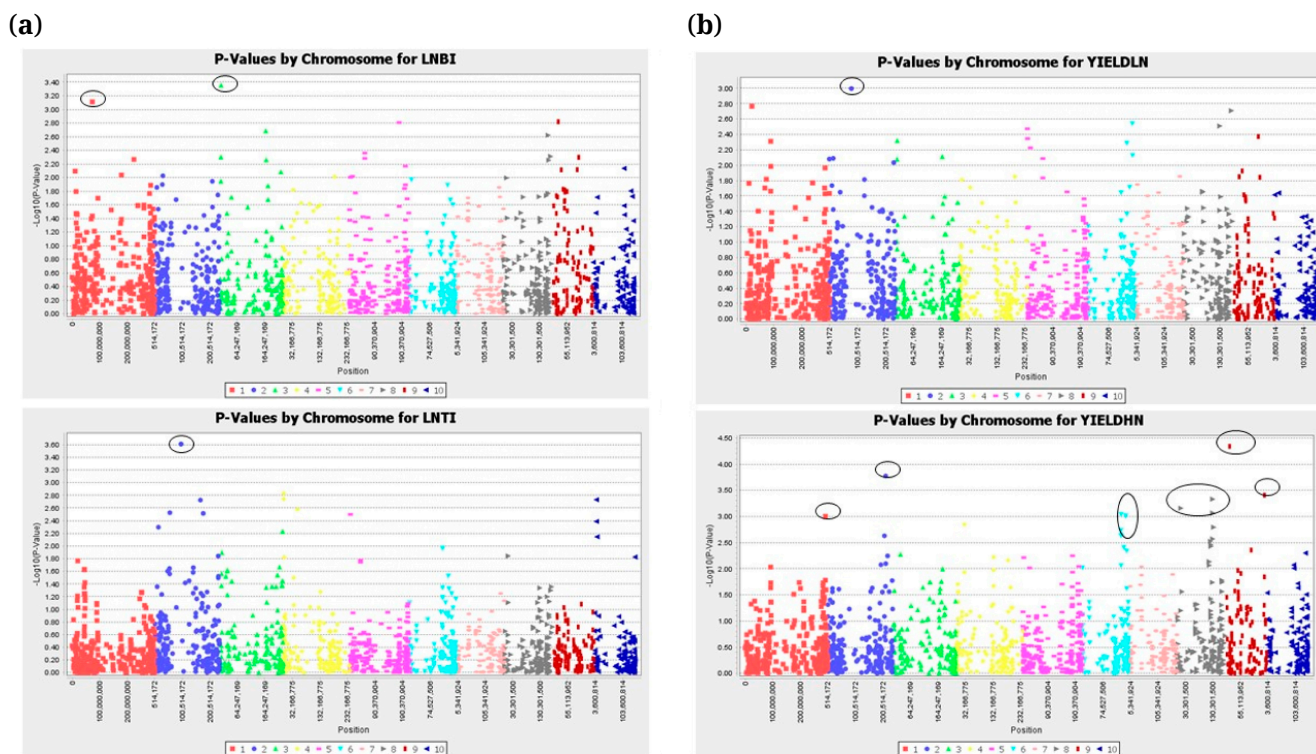
*Genome-Wide Association study of low-soil nitrogen tolerance in early and extra-early maturing quality protein maize inbred lines*

The identification of quantitative trait loci (QTL) conferring tolerance to low-N is crucial for the successful breeding of high-yielding QPM maize genotypes under low-N conditions. We conducted two separate studies with the primary objective of identifying QTLs significantly associated with grain yield and other low-N tolerance-related traits under low-N [3,14]. In the first study, 140 early maturing white QPM inbred lines were evaluated in low-N environments for two consecutive years. The inbred lines were also genotyped with 49,185 DArTseq markers; from which 7599 markers were filtered for population structure analysis and genome-wide association study (GWAS). Results of GWAS identified 24, 3, 10, and 3 significant SNPs respectively associated with grain yield, stay-green characteristic, and plant and ear aspects, under low-N (Table 8). SNP S6\_159734917 was significantly associated with both plant and ear aspects, confirming the positive correlation between the traits, and the possibility of simultaneous improvement of the two traits using the same set of genomic markers. Of the 40 significant SNPs found to be associated with the four measured traits under low-N conditions, 16 were physically located in proximity to 32 putative genes (Table 8). The putative genes *GRMZM2G127139*, *GRMZM5G848945*, *GRMZM2G031331*, *GRMZM2G003493*, *GRMZM2G067964*, *GRMZM2G180254*, on chromosomes 1, 2, 8, and 10 were involved in cellular nitrogen assimilation and biosynthesis, normal plant growth and development, nitrogen assimilation, and disease resistance. The genes identified with different plant biosynthetic mechanisms applicable to maize under low-N could be useful for functional gene studies to clarify the genetic mechanisms underlying low-N tolerance [118].

**Table 8.** List of putative genes associated with four adaptive traits measured under low soil nitrogen.

Traits	Chr	Position	Gene ID	Encoding Products	Functions
Grain yield	2	46273057	GRMZM2G127139	Zeaxanthin epoxidase	Biosynthesis of abscisic acid
			GRMZM2G015610	Protein phosphatase	Unknown function
	1	287891383	GRMZM2G067964	Dolichol-phosphate mannosyltransferase	Plant growth and development
			2	67297792	GRMZM5G848945
	GRMZM5G898735	high chlorophyll fluorescence 106			Photosynthesis
	2	85668156	GRMZM2G049141	E3 ubiquitin-protein ligase UPL3	Unknown function
			2	54250098	GRMZM2G107588
	2	48447873			GRMZM2G157822
			GRMZM2G106108	zinc finger protein CONSTANS-LIKE 1	Plant flowering time
	GRMZM2G028543	putative RING zinc finger domain superfamily protein	Plant growth and development		
	GRMZM2G031331	mlo defense gene homolog 3	Plant disease resistance and leaf cell death		
	2	88084334	GRMZM2G012942	SNARE-interacting protein KEULE	
			7	132400339	GRMZM2G082653
	8	166330750			GRMZM2G003493
			GRMZM2G003518	exocyst complex component EXO70B1	Plant defense response to stress
	GRMZM2G027857	beclin-1-like protein	Cellular response to nitrogen		
	8	164481914	GRMZM2G005290	chitinase CLP	Root and shoot development
			GRMZM2G005433	photosystem II reaction center PSB28 protein	Photosynthesis in plants
	Stay Green Characteristic	10	1417870	AC198366.3_FG004	Scarecrow-like protein 3
GRMZM2G180254				disease resistance protein RGA5	Plant cell death
Plant Aspect	6	159734917	GRMZM2G440849	putative disease resistance protein RGA3	Disease resistance in plant
			GRMZM2G440968	cystatin 3	Unknown function
			GRMZM2G389301	EID1-like F-box protein 3	Plant growth and development
	10	136641842	GRMZM2G169645	putative RING zinc finger domain superfamily protein	Unknown function
			5	8518748	GRMZM2G401040
GRMZM2G065822	WD repeat-containing protein PCN	Leaf formation and development			
Ear Aspect	6	167701917	GRMZM2G375064	GATA transcription factor 25	Chlorophyll biosynthesis
			GRMZM2G375064	glutamate synthase 2 (NADH), chloroplastic	Nitrogen metabolism
	6	159734917	GRMZM2G440849	putative disease resistance protein RGA3	Plant resistance to diseases
			GRMZM2G440968	cystatin 3	Unknown function
	8	169668528	GRMZM2G389301	EID1-like F-box protein 3	Regulates plant growth and development
8	169668528	GRMZM2G171996	protein auxin-regulated gene involved in organ size	Plant organ development	

In the second study, an association mapping analysis was carried out on a collection of 169 extra-early maturing white (50), yellow (53) and orange (57) endosperm inbred lines to reveal the genetic architecture of grain yield and other adaptive traits under low-N environments. The maize collection was phenotyped in three (low-N) and four (high-N) environments during the major growing season in 2018. Out of the 1660, high-quality genotyping by sequencing derived SNP markers used for the genotyping, 11 were significantly associated with grain yield (10 under high-N, and one in low-N environments). Two each were significantly linked to the IITA low-N tolerant base index, which involves yield and five other secondary traits, i.e., ears per plant, anthesis-silking interval, stay green characteristic and plant and ear aspects, and the low-N base index (Figures 5a and 5b). The average phenotypic variation explained (PVE) by these SNPs was 30.01% and three of them had a PVE value greater than 40% (Table 9). These significant associations were delineated into 15 putative candidate genes, majority of which were involved in root functions, probably facilitating nitrogen uptake [119]. Following the validation of the markers and the putative candidate genes, and the SNPs identified in these studies could be used as markers for marker-assisted selection to facilitate genetic gains for low-N tolerance in maize production in SSA.



**Figure 5.** (a) Manhattan plot of marker-trait association analysis for low nitrogen base index (LNBI), low nitrogen tolerance index (LNTI), and (b) grain yield under low-N (YIELDLN) and high-N (YIELDHN).



**Table 9.** List of SNP markers significantly associated with low nitrogen base index (LNBI) low nitrogen tolerance index (LNTI) and grain yield in high-N environments by association mapping.

Trait	Chros		Base		P-value	MAF	Candidate Gene	Gene annotation
	No.	SNP ID	Transition	Position				
LNBI	1	PZE-101084671	A/G	73357546	$7.72 \times 10^{-4}$	0.13	GRMZM2G026137	-
LNBI	3	PZE-103012466	T/C	6650941	$4.42 \times 10^{-4}$	0.36	GRMZM2G380319	Putative MCB2 protein
LNTI	2	PZE-102090548	C/T	96081718	$2.46 \times 10^{-4}$	0.12	GRMZM2G077863	GDSL-like lipase/acylhydrolase putative expressed
LNTI	2	PZE-102090550	C/T	96086002	$2.46 \times 10^{-4}$	0.12	GRMZM2G077863	GDSL-like lipase/acylhydrolase putative expressed
Yield (low-N)	2	PZE-102086253	C/T	79050153	$1.01 \times 10^{-3}$	0.34	AC209972.4_FG005	Protein kinase
Yield (high-N)	1	SYN27559	T/C	291864105	$9.95 \times 10^{-4}$	0.26	GRMZM2G004459	ATP/GTP/Ca <sup>++</sup> binding protein
Yield (high-N)	2	SYN259	T/C	211523915	$1.68 \times 10^{-4}$	0.39	GRMZM2G111809	-
Yield (high-N)	6	PZE-106083594	G/T	141063673	$9.11 \times 10^{-4}$	0.45	GRMZM2G442057	Tetratricopeptide repeat (TPR)-like superfamily protein
Yield (high-N)	6	SYN38086	G/A	158286621	$9.73 \times 10^{-4}$	0.48	GRMZM2G080314	ATBARD1/BARD1 putative expressed
Yield (high-N)	6	SYN38080	T/C	158287119	$9.73 \times 10^{-4}$	0.48	GRMZM2G080314	ATBARD1/BARD1 putative expressed
Yield (high-N)	8	PZE-108014231	C/T	14071576	$6.96 \times 10^{-4}$	0.33	GRMZM2G011213	Mitochondrial glycoprotein family protein
Yield (high-N)	8	PZA01049-1	A/G	129940935	$8.60 \times 10^{-4}$	0.33	GRMZM2G090928	Transmembrane uncharacterized protein
Yield (high-N)	8	PZE-108074836	T/C	129941989	$4.69 \times 10^{-4}$	0.35	GRMZM2G090928	Transmembrane uncharacterized protein
Yield (high-N)	9	PZE-109016384	G/A	16485622	$4.57 \times 10^{-5}$	0.17	GRMZM2G338056	ZOS11-10 - C2H2 zinc finger protein expressed
Yield (high-N)	9	SYN25338	T/C	143003020	$3.93 \times 10^{-4}$	0.21	GRMZM2G150598	ATP-dependent Clp protease ATP-binding protein

Note: Chros = chromosome number; MAF = minor allele frequency; LNBI = low nitrogen tolerance base index.; LNTI = low nitrogen index.

### Summary, Conclusions and Perspectives

This manuscript reviewed the up-to-date literature on tolerance to low-soil nitrogen with a major focus on early and extra-early maturing maize genotypes at IITA. Nitrogen is an important plant nutrient required for growth and productivity but, for several reasons, it is not readily available to the farmers in SSA. Deficiency of nitrogen in maize causes reduced leaf area development, photosynthetic efficiency, kernel weight, ear formation, and accelerated leaf senescence. When bush fallow was prominent in the farming system, there was little attention to N deficiency. With the fallow period disappearing, it has become necessary to find a cheap and sustainable alternative source of N or develop low-N tolerance for maize production. The latter option has been widely adopted by the international research centres (IITA and CIMMYT), in collaboration with NARS of countries in Eastern, Southern, Western, and Central Africa. To begin with, breeders determined that native plus added N should not exceed 30 kg ha<sup>-1</sup> as that required for screening for N tolerance regardless of the differences in soil types, rainfall, and solar radiation received in the different agro-ecological zones in SSA. Therefore, soil samples from low-N must be analysed to ascertain the amount of available N in the soil and to estimate the additional amount of N required to make it up to 30 kg ha<sup>-1</sup>. In addition, the site for screening must be uniform, and measures that improve precision in non-stressed environments will also improve precision in screening for low-N tolerance. Grain yield, which is the primary trait to be improved, and several secondary traits such as flowering (days to 50% anthesis and silk emergence), plant and ear heights, plant and ear aspects, root and stalk lodging, leaf senescence, and ears per plant are measured. Because heritability of grain yield is low under stress factors; selection for grain yield and desirable secondary traits combined in an index selection may be practiced. Germplasm for tolerance to low-N can be screened from diverse sources, including open-pollinated varieties (OPVs) from farmers' fields, elite populations, different types of families (such as S<sub>1</sub>, full-sib and half-sibs), inbred lines, and all types of hybrid varieties. Recurrent selection has been used successfully to screen maize for tolerance to low-N in many programmes of SSA. Using S<sub>1</sub> recurrent selection, two early (TZE-W Pop DT STR C<sub>0</sub> and TZE-Y Pop DT STR C<sub>0</sub>) and two extra-early (TZEE-W Pop DT STR and TZEE-Y Pop DT STR) populations have been improved from which several experimental varieties, inbred lines, and hybrids have been selected for commercialization in SSA. Similar to several investigations of long-term studies in advanced countries, about 50 cultivars generated during three breeding eras (era 1, 1988–2000; era 2, 2001–2006; and era 3, 2007–2011) were evaluated under low and high-N regimes in several environments and definite progress have been made in the third era relative to the two earlier eras. Relative to conventional breeding methods, the molecular approach fast-tracks the development of crop varieties. To date, molecular

efforts toward improving early and extra-early maize for tolerance to low-N at IITA have been the assessment of genetic diversity and population structure, as well as genome-wide association mapping studies. The IITA-MIP is proactively fostering partnerships with CIMMYT and other advanced research institutions to boost its internal capacities, which will ultimately facilitate timely harnessing of emerging technological opportunities. The genetic diversity (GD) and population structure of early and extra-early low nitrogen tolerant maize germplasm have been assessed at IITA using molecular approaches. Genetic distance ranged between 0.042 and 0.500 in one study and from 0.019 to 0.607 in another. A good relationship between combining ability and GD has been reported in several studies. Thus, extensive screening over the years has revealed a wide diversity for low-N tolerance in maize. This milestone offers breeders the opportunity to improve maize germplasm for the trait and release several varieties and hybrids to sustain maize production and alleviate hunger in SSA.

#### **DATA AVAILABILITY**

No data were generated from the study.

#### **AUTHOR CONTRIBUTIONS**

BBA and MAB conceived the review topic. BA, GBA, EOB, PA, AOB and CN conducted the literature search and contributed to the review. CN, BBA and MAB drafted the manuscript. All authors reviewed the final draft.

#### **CONFLICTS OF INTEREST**

The authors declare that there is no conflict of interest.

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