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RESEARCH

Effects of Drought and Low Nitrogen Stress on Provitamin A Carotenoid Content of Biofortified Maize Hybrids

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ABSTRACT

Maize (*Zea mays* L.) hybrids with enhanced provitamin A (proVA) content have been deployed in sub-Saharan Africa, where low soil nitrogen and drought stress are common. The objectives of this study were to assess: (i) the effects of drought and low-N stress on grain proVA content of hybrids with enhanced proVA content, and (ii) the inheritance of proVA carotenoids under these stress conditions. An 11-line diallel cross (55 F_1 crosses) was evaluated for carotenoid content and grain yield under optimum conditions, drought, and low-N stress. Compared with the optimum treatment, mean proVA was lower under both stress treatments. The consistency of genetic effects across stress treatments suggested that hybrids with improved proVA content can be developed for a broad range of environments, provided they are sufficiently adapted. General combining ability (GCA) was significant ($P < 0.01$), and accounted for >85% of the variation among hybrids, whereas specific combining ability (SCA) effects were generally weak ($P < 0.05$), accounting for 5 to 15% of hybrid sums of squares across the three treatments. These results indicated that the inheritance of proVA was not affected by stress treatments. A negative correlation between grain yield and proVA carotenoids was detected, but the data suggested that it was caused by the genetic background of the germplasm used rather than pleiotropy. Our results provide insights that may help breeders design effective breeding strategies to develop proVA-enriched cultivars for resource-limited farming systems.

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Abbreviations: CIMMYT, Centro Internacional de Mejoramiento de Maíz y Trigo (International Maize and Wheat Improvement Center); GCA, general combining ability; HPLC, high-performance liquid chromatography; IITA, International Institute of Tropical Agriculture; MAS, marker-assisted selection; OPC, open-pollinated cultivar; proVA, provitamin A; SCA, specific combining ability.

Maize is a major staple food crop for hundreds of millions of people in sub-Saharan Africa and Latin America, providing more than 30% of the total calories and protein in 11 countries, including Guatemala, Lesotho, Malawi, Zambia, and Zimbabwe (Tanumihardjo et al., 2010; Atlin et al., 2011). Diets of low-income households in some of these countries predominantly consist of maize, which has little or no provitamin A (proVA) carotenoids, and is poor in some micronutrients, such as zinc and iron. Vitamin A deficiency and its associated health issues are highest in some of the countries with high per capita consumption of maize, including Zambia, Malawi, and Zimbabwe (Tanumihardjo et al., 2010; Atlin et al., 2011). Supplementation and industrial fortification programs have failed to reach the most vulnerable populations due to a lack of infrastructure, and the fact that most consumers in developing countries are farmers who produce their own food. Although dietary diversification through on-farm production and consumption of vegetables and fruits is a viable option, that alone

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is ineffective because of the seasonal nature of fruit and vegetable production.

Biofortification, defined as the breeding of staple food crops to increase micronutrient density (Pfeiffer and McClafferty, 2007; Bouis and Welch, 2010; Bouis et al., 2011; Pixley et al., 2013), is a practical and economically viable strategy to fight micronutrient deficiencies in developing countries. HarvestPlus (http://www.harvestplus.org; accessed 11 Feb. 2019) is a program of the Consultative Group on International Agricultural Research (CGIAR) that is involved in breeding crops with increased levels of micronutrients. To combat vitamin A deficiency in sub-Saharan Africa, HarvestPlus and its donors have invested in research, development, and deployment of proVA maize. Improved hybrids that meet 50 to 80% of the breeding target of 15 $\mu g\ g^{-1}$ of proVA have been released in Zambia, Malawi, Zimbabwe, Nigeria, Ghana, the Democratic Republic of Congo, and Tanzania (Pixley et al., 2013; Andersson et al., 2017; Simpungwe et al., 2017), as well as in India (Muthusamy et al., 2014; Zunjare et al., 2018).

In plants, the carotenoid biosynthetic pathway has been well-studied and well-characterized, and the genes involved have been cloned and characterized (Vallabhaneni et al., 2009; von Lintig, 2010; Cuttriss et al., 2011; Wurtzel et al., 2012). The availability of a well-characterized biosynthetic pathway facilitated the identification of genes controlling critical steps in the carotenoid biosynthetic pathway in maize (Harjes et al., 2008; Yan et al., 2010). Selection for favorable alleles at three loci, namely, *phytoene synthase (PSY)*, *lycopene epsilon cyclase* (*LCYε*), and β-*carotene hydroxylase1* (*CRTRB1*), has been effective in increasing proVA content in maize (Li et al., 2008; Muthusamy et al., 2014; Zunjare et al., 2018). The breeding strategy involved selection for increased flux into the carotenoid pathway at *PSY*, and reducing flux into the α branch towards lutein, which has little or no proVA activity, and more into the β side towards β-carotene and β-cryptoxanthin, which have proVA activity (Harjes et al., 2008; Yan et al., 2010; Chandler et al., 2013). Selection for the favorable allele at the *CRTRB1* locus has resulted in four times higher proVA content than that resulting from the wild-type allele (Yan et al., 2010; Babu et al., 2013). Genetic variation at key loci has been exploited through breeding to create sufficient diversity, to enable long-term genetic gain through selection, and reach target levels deemed adequate to impact human nutrition (Azmach et al., 2013; Dhliwayo et al., 2014; Suwarno et al., 2014).

A limited number of studies have been conducted on the combining ability of maize inbred lines for proVA content, with somewhat inconsistent results on the relative importance of additive and nonadditive effects. Egesel et al. (2003) used a diallel of 10 maize inbred lines, and reported that 72 to 87% of the total sum of squares for hybrids for all carotenoids was attributable to general combining ability (GCA). In a different study, Suwarno et al. (2014) used a partial diallel mating design among 21 inbred lines and reported predominance of GCA, with little or no specific combining ability (SCA) effects among 156 hybrids for β-cryptoxanthin and β-carotene. Their data showed that GCA explained 70 (for lutein) to 86% (for proVA) of total hybrid sums of squares. A preponderance of GCA over SCA effects was also reported by Senete et al. (2011), who used a diallel mating design among seven tropical inbred lines, and their results showed that GCA explained 76 to 81% of hybrid sums of squares. In contrast, Halilu et al. (2016) used a diallel mating design of nine inbred lines adapted to West Africa, and reported predominance of nonadditive over additive effects, with GCA effects accounting for 14% (for β-carotene) to 35% (for zeaxanthin) of total hybrid sums of squares.

Breeding and evaluation of proVA hybrids and open-pollinated cultivars (OPCs) are mostly conducted on research farms under optimum agronomic management, including application of the recommended dose of fertilizer and irrigation during extended dry spells in the growing season. Most of the published studies on combining ability for proVA content were also conducted under optimum conditions (Egesel et al., 2003; Senete et al., 2011; Suwarno et al., 2014; Halilu et al., 2016). In contrast, low-income farmers in sub-Saharan Africa grow maize under dryland conditions, with a high frequency and severity of drought (Masih et al., 2014) and with application of limited or no nitrogen fertilizer (Morris et al., 2007). Effects of drought and low nitrogen on grain yield and other agronomic traits are well-documented (Lafitte and Edmeades, 1994; Bolaños and Edmeades, 1996; Bänziger et al., 1999; Trachsel et al., 2016). However, little is known about the effects of drought and low N on proVA content in maize hybrids and OPCs. Such information may be valuable for designing effective breeding strategies to develop hybrids and OPCs targeted for resource-limited farmers in sub-Saharan Africa. The objectives of this study were: (i) to evaluate the effect of drought and low-N stress on proVA content and other carotenoids in biofortified maize hybrids, and (ii) to assess the combining ability of inbred lines for proVA content under low-N and drought stress.

MATERIALS AND METHODS

Experimental Germplasm

Eleven inbred lines (Table 1) varying in proVA content were crossed in a diallel mating scheme (Griffing, 1956), with the seed of reciprocal crosses bulked to form 55 hybrids. Reciprocal crosses were bulked for practical reasons, to obtain a sufficient amount of seed for multi-location testing, and to reduce the cost of both field agronomic trait phenotyping and laboratory carotenoid measurements. The decision to bulk reciprocal crosses was justified by data that show that reciprocal effects are not important for proVA in maize grain (N. Palacios, T. Dhliwayo, and Y. Ortiz-Covarrubias; unpublished data, 2017).

Table 1. Names, pedigrees, provitamin A (proVA) content, and genotypes for 3′ and 5′ ends of the CRTRB1 locus (CRTRB1 3′ and CRTRB1 5′, respectively) and 5′ end of the LYCε locus for the 11 inbred lines crossed in a diallel mating design to form 55 hybrids.

Name†	Pedigree‡	*CRTRB1* 3′§	*CRTRB1* 5′	*LYC*ε 5′	ProVA
					μg g^{-1}
CLHP0020	KUICAROTENOIDSYN-FS17–3-2-B*12	2	2	2	12.98
CHPD1601	(KUICAROTENOIDSYN-FS17–3-2-B-B-B/(KU1409/DE3/KU1409)-S2–18–2-B)-B-4(MAS:L4H1)-2-B*4	1	1	1	32.56
CLHP0022	KUICAROTENOIDSYN-FS25–3-2-B*10	2	2	1	10.42
CHPD1602	(KUICAROTENOIDSYN-FS11–1-1-B-B-B/(KU1409/DE3/KU1409)-S2–18–2-B)-B-2(MAS:L4H1)-2-B*4	1	1	1	25.08
CHPD1603	(KUICAROTENOIDSYN-FS17–3-2-B-B-B/(KU1409/DE3/KU1409)-S2–18–2-B)-B-3(MAS:L4H1)-4-B*5	1	1	1	37.17
CLHP0366	(KUICAROTENOIDSYN-FS17–3-2-B-B-B-B/(CML297/(KU1409/DE3/KU1409)-S2–18–2-B))-B-26–1-B*3	1	1	1	23.90
CLHP0476	(CLQRCWQ97-B///((KUICAROTENOIDSYN-FS17–3-2-B-B-B/(KU1409/DE3/KU1409)-S2–18–2-B)//CAROTENOIDSYN3-FS11–4-3-B-B-B))-B-25–2-B*6	1	1	1	10.38
CLHP0372	(KUICAROTENOIDSYN-FS17–3-2-B-B-B-B/(CML297/(KU1409/DE3/KU1409)-S2–18–2-B))-B-46–3-B*3	1	1	1	10.70
CLHP0068	[[[NAW5867/P30SR]-43–2/[NAW5867/P30SR]-114–1]-9–3-3-B-1-B/CML395–1]-B-13–1-B-4-#/[BETASYN]BC1–8-1–1-1-B*8	2	2	2	7.06
CLHP0003	CML537/[BETASYN]BC1–10–3-#-B*12	2	2	2	11.44
CML451	(NPH28–1/G25//NPH28–1)-2–1-1–3-1-B*6	2	2	2	2.37

† CLHP inbred lines have been selected for both agronomic performance and proVA content; CHPD lines are high proVA donor lines and were not selected for agronomic performance.

‡ In the pedigree B*n (e.g. B*5) represents N times of selfing and shelling selected ears in bulk.

§ The numbers denote the genotype at the locus, where 1 is homozygous for the favorable allele, and 2 is homozygous for the unfavorable allele.

The inbred lines CHPD1601, CHPD1602, and CHPD1603 (Table 1) were developed using marker-assisted selection (MAS) for proVA, targeting the *CRTRB1* and *LYC*ε loci and pedigree selection in biparental populations, with no selection for agronomic traits. The biparental populations from which the three inbred lines were developed involved proVA-enhanced inbred lines from the International Maize and Wheat Improvement Center (CIMMYT) and (KU1409/DE3/KU1409)-S_2–18–2-B, an inbred line obtained from Dr. Abebe Menkir at the International Institute of Tropical Agriculture (IITA). The three inbred lines would be considered first cycle derivatives of the inbred line from IITA, because they were developed from bi-parental populations involving (KU1409/DE3/KU1409)-S_2–18–2-B (Table 1). The lines CLHP0366, CLHP0372, and CLHP0476 would be considered second cycle derivatives, because they were developed by crossing first cycle derivatives of (KU1409/DE3/KU1409)-S_2–18–2-B with adapted high proVA inbred lines (Table 1), and selected for both proVA and agronomic performance. The six inbred lines have the *CRTRB1* proVA favorable allele from the temperate inbred DE3 (Vallabhaneni et al., 2009; Babu et al., 2013) and the *LYC*ε allele (Harjes et al., 2008). Four inbred lines (CLHP0020, CLHP0022, CLHP0003, and CLHP0068) were developed from temperate germplasm with 5 to 8 μg g^{-1} proVA obtained from Dr. Torbert Rocheford and selected for adaptation to tropical environments and for proVA content at CIMMYT (Pixley et al., 2013). The four inbred lines had an average proVA content ranging from 7 to 10 μg g^{-1}, depending on storage conditions and time elapsed after harvest. CML451 is a tropical CIMMYT elite yellow inbred line with excellent yield potential and is also superior in other agronomic traits (Suwarno et al., 2014). The inbred line CML451 had proVA content averaging 2 to 4 μg g^{-1}, which is within the normal range for yellow maize inbred lines that have not been enhanced for proVA content. Information on the genotype of each of the 11 inbred lines (Table 1) at the *CRTRB1* (both the 3′ indel and 5′TE alleles) and the *LYC*ε (Lcyε-5′TE) loci (Harjes et al., 2008; Yan et al., 2010) was obtained from data that were generated for MAS for proVA in our breeding program.

Field Experiment Layout and Agronomic Management

The 55 F_1 hybrids and a single-cross hybrid check (CML451 × CML486) were evaluated using an α-lattice (0,1) design (Patterson et al., 1978) with two replications at three locations. The trial was planted under optimum and low-N conditions at CIMMYT's research station near Agua Fria, Puebla, Mexico (20° 27′ N 1, 97° 38′ W; 110 masl) during winter season (Nov.–May) in 2013 and at the CIMMYT research station near Harare, Zimbabwe (17°43′ S, 31° 01′ W; 1506 masl) during the summer (Nov.–May) of 2014. Drought-stress experiments, and their corresponding optimum trials, were grown in the same block, but were separated by six border rows at Tlaltizapán, Morelos, Mexico (18°41′ N, 99° 07′ W; 945 masl) during the winter seasons in 2013 and 2014. The experiment was therefore planted in two low-N stress, two drought-stress, and four optimum environments. Plot size at Agua Fria and Tlaltizapán was two 5-m rows, with 0.75 m between rows and 0.15 m between plants within a row, for a final plant population of 88,000 plants ha^{-1}. Plot size at Harare was a single 4-m row, with 0.75 m between rows and 0.25 m between plants within a row, for an effective plant population of 54,000 plants ha^{-1}.

The optimum-input trials at each location were managed using the recommended agronomic practices. At Agua Fria and Tlaltizapan, 100 kg N ha^{-1} were applied as ammonium sulfate before planting, followed by another 100 kg N ha^{-1} applied as urea

30 to 35 d after planting. In Zimbabwe, 25 and 112 kg N ha^{-1} were applied before planting and at 6 wk after planting, respectively. Drip irrigation was applied on a weekly basis at Tlaltizapán and Agua Fria to compensate for evapotranspiration and to avoid moisture stress at any stage during the growing season. Depending on soil type, crop growth stage, temperature, and evapotranspiration, 20 to 60 mm of water was applied every week up to physiological maturity. In Harare, the optimum-input trial was grown under rainfed conditions, but water was applied via sprinkler irrigation at the first sign of moisture stress (e.g., leaf rolling).

To simulate maize-growing conditions of smallholder farmers in sub-Saharan Africa, we conducted low-N experiments in fields that had been depleted of N for at least five previous cropping seasons. Nitrogen depletion was achieved by growing non-leguminous crops without applying N fertilizer, and then cutting and removing the biomass after each season (Bänziger et al., 1999; Bänziger et al., 2000; Trachsel et al., 2016). The low-N trials were managed following recommended agronomic practices, except that no N fertilizer was applied. Irrigation scheduling for low-N trials was done as described for the optimum experiments in both Mexico and Zimbabwe. Low-N and their corresponding optimum-input experiments were grown in separate blocks at Agua Fria and Harare.

Drought trials at Tlaltizapán were managed following methods described by Trachsel et al. (2016). Briefly, the last irrigation of about 40 mm was applied at 12 to 15 d before flowering, targeting to reach the permanent wilting point at a soil depth of 30 to 40 cm at flowering. Additional irrigation was applied 14 d after anthesis to ensure good grain filling. Soil moisture content was monitored for all trials throughout the cropping cycle with Delta-T soil moisture probes (Delta-T Devices, Cambridge, United Kingdom). Moisture data were used in conjunction with field observations for irrigation scheduling. Use of drip irrigation allowed for precise application of water, such that planting the trials in separate blocks was not necessary.

For all trials, the first and last plant of each row were self-pollinated to produce F_2 grain for carotenoid analysis. Four plants were self-pollinated in 2-row plots at Agua Fria and Tlaltizapán, whereas two plants were self-pollinated in single-row plots at Harare. End-plants were self-pollinated to collect samples for proVA analysis for all environments, and plots to ensure that any bias attributable to border effects was the same for all plots. In addition, we wanted to avoid use of end-plants to collect grain yield data, which we presumed to be more responsive to border effects than carotenoids based on repeatability estimates from previous studies (Menkir and Maziya-Dixon, 2004; Dhliwayo et al., 2014; Suwarno et al., 2014).

Field Data Collection

Data were collected on a plot basis for grain yield in Mg ha^{-1} (adjusted to 125 g kg^{-1} moisture content), number of days to anthesis (number of days from planting to 50% pollen shed), and plant height in cm (average distance from soil surface to the base of the flag leaf). Grain yield data were collected on the remaining open-pollinated plants after harvesting the self-pollinated ears.

Carotenoid Analyses

At harvest, a balanced bulk of 100 kernels from 2 to 4 self-pollinated ears per plot was formed and used for carotenoid analyses. The grain was promptly dried and transferred to cold storage (−80°C) to minimize carotenoid losses prior to conducting analyses. Samples of 20 to 30 kernels per plot were taken from the bulk and used for the analyses.

The analyses were performed, as described by Babu et al. (2013). Briefly, ethanol was added to ground maize grain samples to release carotenoids. The samples were then saponified, followed by extraction of carotenoids using hexane before separation and quantification using HPLC with a 30C column attached to a YMC C30 filter insert. A multi-wavelength detector set to 450 nm was used, and data were collected and processed using Waters Millennium 2010 software (Waters Chromatography, Milford, MA). Lutein, zeaxanthin, β-cryptoxanthin, and all trans-β-carotene were identified through their characteristic spectra and comparison of their retention times with known standard solutions. Total proVA content (μg g^{-1}) was calculated for each sample on a dry weight basis as the sum of β-carotene plus one-half of β-cryptoxanthin.

Statistical Analyses

Analysis of variance (ANOVA) was conducted for each treatment using PROC GLM (SAS Institute, 2008), according to the following model:

$$Y_{ijkl} = \mu + \alpha_l + b_{k(l)} + \nu_{ij} + (\alpha\nu)_{ijl} + e_{ijkl} \quad [1]$$

$$\nu_{ij} = g_i + g_j + s_{ij} \quad [2]$$

where Y_{ijkl} = observed trait value for each plot, μ = the grand mean, α_l = location or environment effect, $b_{k(l)}$ = block effect nested within environment, ν_{ij} = F_1 hybrid effect = $g_i + g_j + s_{ij}$ (where g_i = the GCA of the ith parent, g_j = GCA of the jth parent, and s_{ij} = SCA of the ijth hybrid), $(\alpha\nu)_{ijl}$ = the interaction between the lth environment and the ijth F_1 hybrid, and e_{ijkl} = residual effect or experimental error. Hybrid effects were considered fixed, whereas environment (location) and block effects were considered random. Estimates of repeatability across environments were calculated for each treatment, as described by Hallauer et al. (2010), using the formula:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \left(\sigma_{gl}^2 / l\right) + \left(\sigma_e^2 / rl\right)}$$

where σ_g^2 is the genotypic variance, σ_{gl}^2 is the genotype by environment interaction variance, σ_e^2 is error variance, r is the number of replications and l is the number of environments. The diallel analysis for each stress treatment was conducted using DIALLEL-SAS05 program (Zhang et al., 2005) for Griffing's Model 1 and Method 4 (Griffing, 1956). F-tests for hybrid effects and their sub-partitions (GCA and SCA) were done using their respective interactions with environment as the error variance, whereas interactions with environment were tested for significance using the residual variance as the error term.

Because of the large differences in planting density, the Zimbabwe environments were first analyzed separately, and Spearman's rank correlation coefficients were calculated to assess the degree of relationship and feasibility for conducting combined statistical analyses with Mexico locations. The optimum environments in Zimbabwe and Mexico did not show significant rank correlation for grain yield ($-0.02 \leq r \leq 0.09$), possibly because of a large error variance and low

Table 2. Mean squares for grain yield (GY), β-carotene (BC), β-cryptoxanthin (BCX), lutein, and zeaxanthin (ZX) for 55 hybrids from a diallel mating design evaluated under optimum conditions in three environments for grain yield, and four environments for carotenoid traits. Grain yield was measured in Mg ha^{-1} and carotenoids were measured in μg g^{-1}.

		Mean squares					
Source†	DF‡	GY	ProVA	BC	BCX	Lutein	ZX
Environments, E	2/3	39.48**	287.4**	81.9**	63.2**	3.99**	25.1**
Replications/E	3/4	8.24**	6.1	3.6	0.9	0.92	4.1
Hybrids, H	54	2.88**	235.0**	162.9**	47.5**	35.05**	302.2**
GCA	10	9.31	1204.0**	830.5**	228.2**	164.19**	1418.8**
SCA	44	1.42*	14.8**	13.1**	6.4*	5.70**	48.4**
H × E	108/162	1.52**	7.6**	5.4**	1.6**	1.07**	9.4**
GCA × E	20/30	4.40**	13.8**	9.3**	2.7**	1.26**	13.5**
SCA × E	88/132	0.87**	5.9*	4.2*	1.4**	0.97*	7.9*
Error	162/216	0.49	4.59	3.20	0.84	0.74	5.93
Repeatability		0.57	0.97	0.97	0.97	0.97	0.97
GCA SS/H SS§		0.60	0.95	0.94	0.89	0.87	0.87

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

† GCA, general combining ability effects; SCA, specific combining ability effects.

‡ DF, degrees of freedom; "n_1/n_2" indicates degrees of freedom for grain yield (n_1, for three environments) and for carotenoids (n_2, for four environments), respectively.

§ Additive effect (GCA) sum of squares as a proportion of total hybrid sum of squares.

heritability observed in Zimbabwe. As a result of this, grain yield data obtained in Zimbabwe was excluded from the combined analysis. The Spearman's rank correlation coefficient between Zimbabwe and Mexico under low-N environments was moderate to high ($r > 0.6$, $P < 0.01$), suggesting that combined analysis was justified. Pearson's phenotypic correlation coefficients were then calculated for carotenoids and other agronomic traits to assess trends in trait responses among optimum, drought, and low-N treatments.

RESULTS AND DISCUSSION

Carotenoid Variances

Despite the large differences in planting density between Zimbabwe and Mexico environments, carotenoid concentrations were highly correlated across environments, with correlation coefficients ranging from $r = 0.82$ ($P < 0.01$) for zeaxanthin under optimum conditions to $r = 0.94$ ($P < 0.01$) for lutein under low-N conditions. The high correlation coefficients suggested that differences in planting density and agronomic management had little or no influence on carotenoid concentration.

Under optimum conditions, GCA effects were highly significant ($P < 0.01$) for proVA and all carotenoids. SCA effects under optimum conditions were highly significant ($P < 0.01$) for proVA, β-carotene, lutein, and zeaxanthin, with β-cryptoxanthin being only significant at $P < 0.05$ (Table 2). Repeatability estimates were high for proVA and other carotenoids, averaging 0.97 for each of the five variables. The contribution of GCA sum of squares to total hybrid sum of squares averaged 95, 94, 89, 87, and 87% for proVA, β-carotene, β-cryptoxanthin, lutein, and zeaxanthin, respectively (Table 2), consistent with findings from some studies (Egesel et al., 2003; Senete et al., 2011; Suwarno et al., 2014) but differed from the report of Halilu et al. (2016), who found a greater influence of non-additive effects on the same carotenoids. The greater contribution of GCA effects in this study compared with previous studies was not surprising because several lines were genetically related (Table 1). With related inbred lines, hybrids are expected to have less dominance because they share common alleles at several loci (Hallauer et al., 2010). This might explain why our results differed from those of Halilu et al. (2016), who used a more diverse set of lines for their study.

Highly significant hybrid and GCA effects were found under drought and low-N stress environments (Table 3). The SCA effects for proVA and β-carotene were not significant under drought stress and only significant at $P < 0.05$ under nitrogen stress. In contrast, SCA effects for β-cryptoxanthin were significant under drought stress ($P < 0.05$) and under low-N ($P < 0.01$) conditions. The SCA effects for lutein and zeaxanthin were not significant under drought stress but were highly significant ($P < 0.01$) under low-N stress conditions. Although the contribution of GCA sum of squares to total hybrid sum of squares was lower under stress than under optimum conditions, ranging from 84% for zeaxanthin under drought to 94% for proVA under low-N stress, the results still showed a preponderance of GCA effects over SCA effects for all carotenoids and proVA (Table 3).

The hybrid × environment (H × E) interaction was significant ($P < 0.05$) for proVA, β-carotene, β-cryptoxanthin, and lutein under both drought stress and low N, and for zeaxanthin only under low N (Table 3). Partitioning of the H × E sum of squares into its subcomponents revealed several significant interactions of GCA and SCA with environments that were less consistent when compared with the main effects (Table 3). The contribution of H × E interaction

Table 3. Mean squares for grain yield, provitamin A (ProVA), β-Carotene, β-Cryptoxanthin (BCX), lutein, and zeaxanthin (ZX) under drought stress (DS) and low-nitrogen (LN) conditions. Grain yield was measured in Mg ha^{-1} and carotenoids were measured in mg g^{-1}.

		Grain Yield		ProVA		β-Carotene		BCX		Lutein		ZX	
Source	DF†	DT	LN	DS	LN	DS	LN	DS	LN	DS	LN	DS	LN
Environments, E	1	0.6ns	301.3**	951.3**	1206.9**	371.0**	572.4**	105.8**	29.9**	0.2	25.2**	0.7	69.9**
Replications/E	2	0.7*	0.7	21.1**	3.6*	9.3**	4.6**	1.4	1.3**	0.3	0.7	0.9	2.7
Hybrids (H)	54	0.5**	1.6**	105.2**	58.8**	70.9**	41.9**	31.3**	15.5**	13.5**	15.5**	88.1**	123.9**
GCA‡	10	1.5*	6.9	493.3**	298.9**	324.8**	208.2**	146.7**	73.2**	61.7**	71.4**	400.7**	581.4**
SCA§	44	0.3	0.3	16.6	5.1*	13.3	4.4**	5.5*	1.9**	2.6	2.5**	17.5	17.5**
H × E	54	0.2	0.7	24.6**	4.0**	16.5**	2.7**	3.9**	1.2**	1.7*	0.9**	11.5	6.3**
GCA × E	10	0.4	2.4**	53.3**	0.7	33.4**	0.0	8.2**	2.7**	1.1	1.4**	11.4	7.4**
SCA × E	44	0.2	0.3	16.1**	2.3**	11.3**	1.4**	2.8	0.6**	1.7*	0.5	11.1	4.8**
Error	108	0.2	0.5	3.01	1.16	2.26	0.86	2.33	0.29	1.1	0.4	8.2	2.76
Repeatability		0.59	0.56	0.78	0.93	0.77	0.94	0.88	0.92	0.88	0.94	0.88	0.95
GCA SS/H SS¶		0.52	0.82	0.87	0.94	0.85	0.92	0.87	0.87	0.85	0.85	0.84	0.87

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

† DF, degrees of freedom.

‡ GCA, general combining ability effects.

§ SCA, specific combining ability effects.

¶ Additive effect (GCA) sum of squares as a proportion of total hybrid sum of squares.

to the total sum of squares varied from 0.03 to 0.36% under stress and was considered too small to be of practical significance. These results are consistent with those of previous studies that reported little or no interaction for carotenoid concentration and proVA content in maize grain (Egesel et al., 2003; Menkir and Maziya-Dixon, 2004; Dhliwayo et al., 2014; Suwarno et al., 2014; 2015). Taken together, the high heritability estimates and the consistency in the relative importance of additive to non-additive effects under all treatments suggested that the inheritance of carotenoids was not affected by the stress treatments.

Carotenoid Means

The higher diallel hybrid means for proVA and lack of significant difference for lutein and zeaxanthin, relative to the normal check hybrid, under all treatments suggested that progress had been made in improving proVA content without affecting the non-target carotenoids. The mean for diallel hybrids was higher under each stress treatment and across all stress treatments than that of the normal yellow check hybrid, CML451 × CML486, for proVA (12.90 vs. 4.08 μg g^{-1}), β-carotene (7.57 vs. 1.30 μg g^{-1}), and β-cryptoxanthin (3.94 μg g^{-1} vs. 3.46 μg g^{-1}). The diallel hybrid means for zeaxanthin (7.54 μg g^{-1}) and lutein (2.92 μg g^{-1}) were not significantly different from that of the check hybrid (7.37 μg g^{-1} and 3.0 μg g^{-1}, respectively). Mean proVA content of hybrids across stress treatments was 12.90 μg g^{-1}, with individual hybrid means ranging from 3.53 μg g^{-1} for CML451 × CLHP0068 to 22.62 μg g^{-1} for CHPD1603 × CHPD1601. These two hybrids also had the lowest (1.39 μg g^{-1} for CML451 × CLHP0068) and highest (16.34 μg g^{-1} for CHPD1603 × CHPD1601) values for β-carotene content, with a mean of 7.57 μg g^{-1}. The means for β-cryptoxanthin were lower than those for β-carotene, ranging from 0.74 μg g^{-1} for CHPD1602 × CHPD1601 to 10.50 μg g^{-1} for CLHP0020 × CLHP0022, with a mean of 3.94 μg g^{-1}.

Although variation in methods and conditions can affect results for different studies, the range for lutein (0.45–8.06 μg g^{-1}) was lower than that reported in other studies for both tropical and temperate maize (Kurilich and Juvik, 1999; Ortiz-Monasterio et al., 2007; Harjes et al., 2008; Menkir et al., 2008). The reduced lutein concentration may be attributed to selection that favored more flux into the β branch than into the α branch of the carotenoid pathway (Harjes et al., 2008; Meier et al., 2011; Wurtzel et al., 2012; Babu et al., 2013; Pixley et al., 2013; Dhliwayo et al., 2014).

Both β-carotene and β-cryptoxanthin were equally responsive to drought and low-N stress. However, drought stress enhanced β-cryptoxanthin levels and decreased β-carotene content, whereas low-N stress decreased the levels of both carotenoids (Table 4). Lutein and zeaxanthin, on the other hand, were the more stable carotenoids under both drought stress and low-N stress.

Means for the three stress treatments showed that proVA was highest (14.1 μg g^{-1}) under optimum conditions, followed by drought stress (12.9 μg g^{-1}), and lowest under low-N stress (10.7 μg g^{-1}; Table 4). The lower proVA content under stress resulted from reductions in constituent carotenoids β-carotene and β-cryptoxanthin (Table 4). Relative to optimum conditions, β-carotene decreased by 16.5% under drought stress and by 26.5% under low-N stress. In contrast, β-cryptoxanthin increased by 22.4% under drought stress and decreased by 20.6% under low-N stress. Lutein also increased by 9.6% under drought stress

Table 4. Trait means and their standard errors under optimum conditions, drought stress, and low nitrogen stress.

Trait†	Optimum	Drought	Low Nitrogen
Grain yield, Mg ha^{-1}	4.63 ± 0.04	0.96 ± 0.03	2.50 ± 0.04
Anthesis date, d	79.9 ± 0.09	75.8 ± 0.08	82.8 ± 0.09
ASI, d	0.78 ± 0.07	2.00 ± 0.09	1.79 ± 0.08
Moisture, %	13.2 ± 0.05	10.2 ± 0.02	12.6 ± 0.05
Plant height, cm	186. ± 0.59	157.0 ± 0.57	142.0 ± 0.80
ProVA, μg g^{-1}	14.1 ± 0.04	12.9 ± 0.11	10.7 ± 0.07
β-Carotene, μg g^{-1}	8.53 ± 0.12	7.12 ± 0.10	6.27 ± 0.06
β–Cryptoxanthin, μg g^{-1}	3.93 ± 0.06	4.81 ± 0.10	3.12 ± 0.03
Lutein, μg g^{-1}	2.94 ± 0.05	3.19 ± 0.07	2.65 ± 0.04
Zeaxanthin, μg g^{-1}	7.97 ± 0.16	7.52 ± 0.19	7.22 ± 0.11

† ASI, anthesis-silking interval; proVA, provitamin A content.

and decreased by 9.8% under low-N stress. Zeaxanthin was the least affected by stress, with a decrease of 5.6% under drought stress and 9.4% under low-N stress.

Limited information is available on the effects of stress on carotenoid metabolism, and we, therefore, cannot adequately explain why there was a reduction in β-carotene and β-cryptoxanthin. One possible explanation is that carotenoids form part of the plant's response mechanism to stress (Altangerel et al., 2017) and thus are partitioned towards systems that stimulate tolerance to stress and less to the grain. Increases in plant carotenoid concentrations under drought stress have previously been reported for pepper, tomato, and sweet potato (Wang and Frei, 2011). Similarly, Guzmán et al. (2016) reported increased yellowness in wheat attributable to an increase in carotenoids in grain produced under drought stress.

Carotenoid GCA Effects

Estimates of GCA effects showed that all inbred lines having the favorable allele at the *CRTRB1* and *LYC*ε loci (CHPD1601, CHPD1602, CHPD1603, CLHP0366, CLHP0372, and CLHP0476) had positive and mostly significant GCA effects for proVA and β-carotene under optimum, drought stress, and low-N stress conditions (Table 5). However, some of these lines had significant and negative GCA effects for β-cryptoxanthin, indicating that the high GCA effects for proVA were mostly associated with higher concentration of β-carotene. The favorable *CRTRB1* allele reduces hydroxylation of β-carotene to β-cryptoxanthin and is more effective at increasing proVA content than the favorable allele of *LYC*ε that reduces flux into the α branch of the pathway (Harjes et al., 2008; Babu et al., 2013). It is not surprising therefore that inbred lines that had the favorable allele at the *CRTRB1* locus tended to have insignificant to negative GCA effects for β-cryptoxanthin (Table 5), a finding which is confirmed by moderate negative correlations between β-carotene and β-cryptoxanthin across all three treatments (Table 6).

Of the other five inbred lines that did not have the favorable allele at the *CRTRB1* locus, only CLHP0003 (under optimum conditions) and CML451 (under all stress treatments) had a negative and highly significant ($P < 0.01$) GCA effect for β-cryptoxanthin. CLHP0003 is a first-cycle yellow line derived from a white inbred line CML537, whereas CML451 was developed from a population segregating for white and yellow kernel color. The two inbred lines have pale yellow grain, as opposed to deep yellow to orange color of the other nine lines. Although grain color is not correlated with proVA content, it is moderately correlated with total carotenoids (Harjes et al., 2008). It is thus possible that CML451 lacked favorable alleles at key loci in the carotenoid pathway, which led to the observed negative GCA effects for β-cryptoxanthin.

The GCA effects for all carotenoids were mostly stable across stress treatments, with a few exceptions where changes in magnitude of effects were detected, such as CLHP00476 for proVA, and CLHP0366 and CLHP072 for β-cryptoxanthin (Table 5). The stable effects across stress treatments also allowed the identification of key lines that breeders could use as source germplasm for improving specific carotenoids. For example, CLHP0020 and CLHP0022 would be good sources of β-cryptoxanthin, lutein, and zeaxanthin across stress treatments, whereas the lines CHPD1601, CHPD1602, CHPD1603, and CLHP0372 would be the best germplasm sources for proVA and β-carotene across stress treatments.

Grain Yield

Hybrid effects were significant ($P < 0.01$) for grain yield under all three stress treatments (Tables 3, 4). Mean grain yield was highest under optimum conditions (4.63 Mg ha^{-1}), followed by low-N stress (2.50 Mg ha^{-1}), and was lowest under drought stress (0.96 Mg ha^{-1}). Although 60% of the hybrid sum of squares for grain yield under optimum conditions was attributed to additive effects, the GCA effects were not significant ($P = 0.055$), whereas SCA effects, which explained 40% of the hybrid sum of squares, were highly significant ($P < 0.01$). The GCA effects for grain yield were significant ($P < 0.05$) under drought stress, but not under low-N conditions. In contrast, SCA effects were not significant under both low-N and drought stresses (Table 3). Repeatability estimates of 0.57 under optimum conditions, 0.59 under drought stress, and 0.56 under low-N stress (Tables 3, 4) can be considered moderate when compared with values reported in the literature (Hallauer et al., 2010). These repeatability estimates were consistent with previous studies, which reported lower estimates for grain yield than for carotenoids (Dhliwayo et al., 2014; Suwarno et al., 2014), implying that fewer environments would be required to evaluate carotenoid content, and thus allowing breeders to reduce the cost of HPLC phenotyping (Zhang et al., 2012).

The GCA effects for CLHP0068 were significant ($P < 0.05$) under optimum conditions and drought stress, whereas those for CLHP0003 were significant ($P < 0.05$) under optimum conditions and low-N stress. In contrast,

Table 5. General combining ability effects of inbred lines for grain yield, provitamin A, β-carotene, β-cryptoxanthin, lutein, and zeaxanthin under optimum conditions, drought stress, and low-nitrogen stress.

	Grain Yield			Provitamin A			β-carotene		
	Mg ha^{-1}			μg g^{-1}					
Inbred Line	OPT†	DS	LN	OPT	DS	LN	OPT	DS	LN
CLHP0020	−0.64**	−0.22**	−0.32	−1.66**	−1.59**	−1.21**	−2.73**	−2.53**	−1.92**
CHPD1601	−0.56**	−0.10	−0.25	4.95**	4.27**	3.06**	4.65**	3.63**	2.76**
CLHP0022	−0.25	−0.02	−0.36	−0.89**	−1.24*	−0.89	−2.30**	−2.16**	−1.87**
CHPD1602	0.01	−0.09	−0.33	3.30**	4.05**	2.18**	3.59**	3.85**	2.21**
CHPD1603	−0.06	−0.12	−0.16	4.72**	4.30**	3.28**	3.80**	3.32**	2.70**
CLHP0366	−0.04	−0.13	−0.13	2.49**	2.10**	2.28**	2.37**	1.55**	1.93**
CLHP00476	0.19	0.05	0.17	0.56	0.94	1.03*	1.03**	1.85**	1.39**
CLHP00372	−0.10	−0.15*	−0.49*	2.30**	1.00	1.45**	2.09**	0.484	1.11**
CLHP0068	0.28*	0.46**	0.43	−4.99**	−3.22**	−3.65**	−4.11**	−2.83**	−2.99**
CLHP0003	0.31*	0.06	0.69**	−2.84**	−3.30**	−1.77**	−2.52**	−2.63**	−1.42**
CML451	0.85**	0.26**	0.75**	−7.93**	−7.32**	−5.75**	−5.87**	−4.51**	−3.89**
V(g_i)‡	0.27	0.10	0.25	0.42	1.16	0.14	0.34	0.92	(−0.70)
V(g_i−g_j)§	0.40	0.14	0.37	0.62	1.72	0.20	0.51	1.36	(−0.70)
	β-Cryptoxanthin			Lutein			Zeaxanthin		
	μg g^{-1}								
CLHP0020	3.36**	3.48**	2.53**	2.91**	2.29**	2.55**	8.53**	6.00**	7.41**
CHPD1601	−1.37**	−1.31**	−0.95**	−0.51**	−0.53**	−0.47**	−1.73**	−1.50**	−1.63**
CLHP0022	4.32**	3.19**	2.91**	2.32**	1.93**	2.45**	7.72**	4.88**	7.06**
CHPD1602	−1.91**	−2.14**	−1.25**	−0.85**	−0.74**	−0.85**	−2.15**	−1.73**	−2.02**
CHPD1603	−0.09	−0.17	−0.52**	−0.30**	−0.32	−0.35**	−0.44	−0.57	−1.08**
CLHP0366	−0.69**	−0.30	−0.64**	−0.10	−0.16	−0.18	−0.81**	−0.62	−0.95**
CLHP00476	−2.25**	−2.65**	−1.50**	−2.23**	−2.35**	−2.05**	−6.16**	−5.67**	−5.75**
CLHP00372	−0.21	0.74**	−0.05	0.88**	0.94**	0.78**	1.66**	2.41**	2.03**
CLHP0068	0.25	0.97**	0.37**	−0.45**	0.13	−0.48**	−1.07**	0.26	−0.89**
CLHP0003	−0.43**	0.14	0.05	−1.41**	−1.01**	−1.26**	−4.47**	−2.95**	−3.66**
CML451	−0.98**	−1.94**	−0.96**	−0.24*	−0.20	−0.14	−1.09**	−0.51	−0.52
V(g_i)¶	0.235	0.46	0.26	0.13	0.17	0.19	0.41	0.54	0.43
V(g_i−g_j)#	0.349	0.68	0.39	0.19	0.25	0.28	0.61	0.80	0.64

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

† OPT, estimates of general combining ability under optimum conditions; DS, estimates of general combining ability under drought stress; LN, estimates of general combining ability under low nitrogen stress.

‡ Variance of general combining ability effects.

§ Variance of the difference between general combining ability effects of two inbred lines.

CML451 had significant ($P < 0.01$) GCA effects under all three treatments (Table 5). These three lines are currently being used as testers or parents of new populations to develop new inbred lines in the HarvestPlus proVA and CIMMYT's subtropical and tropical breeding programs. CLHP0003, in particular, has been used as a parent in several hybrids released in Zambia, Zimbabwe, Malawi, and Tanzania. On the other hand, CML451 is one of the best yellow inbred lines and has been used to produce successful commercial hybrids both at CIMMYT and in other breeding programs in the tropics and subtropics.

The inbred CLHP0020 had significant ($P < 0.01$) negative GCA effects for grain yield under drought stress and optimum conditions (Table 5), whereas CLHPD1601 had a significant ($P < 0.01$) negative GCA effect only under optimum conditions. CLHP0372 had significant ($P < 0.01$) negative GCA effects for grain yield under drought and low-N stress. In general, most lines that had positive GCA effects for grain yield had significant and negative GCA effects for proVA, except CLHP0020, which had significant and negative GCA effects for both grain yield and proVA (Table 5). These results are further confirmed by significant ($P < 0.01$) negative correlation coefficients between grain yield and proVA under all growing conditions ($r \leq -0.56$; Table 6). The negative correlation coefficients between grain yield and proVA are probably a result of linkage drag, rather than pleiotropy. The germplasm used to develop high proVA inbred lines, such as CLHP0366, CLHP0372, and CLHP0476, were developed using MAS for *CRTRB1* and *LYCε*, with little or no selection for agronomic traits. Haplotype blocks associated with high proVA carotenoids from the temperate source germplasm might have increased

Table 6. Pearson's phenotypic correlation coefficients among grain yield (GY, Mg ha^{-1}), number of days to anthesis (AD), grain moisture (MOI, %), plant height (PH), lutein (μg g^{-1}), zeaxanthin (ZX, μg g^{-1}), β-cryptoxanthin (BCX, μg g^{-1}), β carotene (BC, μg g^{-1}), and provitamin A (proVA, μg g^{-1}) under optimum conditions, drought stress, and low nitrogen stress.

OPT/DS†	GY	AD	MOI	PH	Lutein	ZX	BCX	BC	proVA
GY	–	−0.45**	0.37**	0.62**	−0.23	−0.24	−0.11	−0.50**	−0.58**
AD	−0.46**	–	−0.08	−0.28*	0.15	0.12	−0.17	0.47**	0.49**
MOI	−0.25	0.03	–	0.27	−0.1	−0.11	−0.07	0.12	0.1
PH	0.29*	−0.22	−0.18	–	−0.51**	−0.51**	−0.42**	−0.06	−0.17
Lutein	−0.01	−0.09	0.14	−0.39**	–	0.99**	0.85**	−0.28*	−0.16
ZX	−0.01	−0.09	0.16	−0.39**	0.99**	–	0.88**	−0.29*	−0.17
BCX	0.01	−0.32**	0.06	−0.2	0.86**	0.86**	–	−0.45**	−0.32**
BC	−0.47**	0.42**	0.14	−0.16	−0.39**	−0.37**	−0.39**	–	0.98**
proVA	−0.56**	0.42**	0.19	−0.28*	−0.21	−0.19	−0.2	0.96**	–
Low N‡	**GY**	**AD**	**MOI**	**PH**	**Lutein**	**ZX**	**BCX**	**BC**	**proVA**
GY	1								
AD	−0.1	1							
MOI	0.63**	−0.1	1						
PH	0.56**	0.63**	0.04	1					
Lutein	−0.32**	−0.05	−0.02	−0.47**	1				
ZX	−0.32**	−0.09	−0.03	−0.49**	0.99**	1			
BCX	−0.14	−0.29*	0.07	−0.29*	0.82**	0.84**	1		
BC	−0.51**	0.12	−0.59**	−0.04	−0.36**	−0.38**	−0.47**	1	
proVA	−0.63	0.12	−0.64**	−0.14	−0.23	−0.25	−0.32**	0.97**	1

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

† Correlation coefficients under optimum (OPT) conditions and under drought stress (DS), above and below diagonal, respectively.

‡ Correlation coefficients among traits under low nitrogen stress.

proVA concentration, but they might also have been in linkage disequilibrium with other alleles that compromised adaptation and agronomic performance, including yield. Selection for increased grain yield and other agronomic traits during the development of CLHP0366, CLHP0372, and CLHP0476 meant that carotenoid content had to be compromised, and this may explain why these second-cycle derivatives of the inbred line obtained from IITA had lower proVA content than the first-cycle derivatives (Table 1).

The second probable cause of the high and negative GCA effects for grain yield of inbred lines with high proVA content is that these lines were derived from a common parent (Table 1), resulting in low heterosis for grain yield for their hybrid combinations. The inbred lines CML451, CLHP0003, and CLHP0068 were the most unrelated (Table 1), and therefore expressed the most heterosis for grain yield when crossed with the rest of the lines. Still, the fact that second-cycle derivatives of the source germplasm obtained from IITA (e.g., CLHP0366, CLHP0476, and CLHP0372) tended to have nonsignificant and negligible negative GCA effects for grain yield, suggests that it is possible to increase both grain yield and proVA concentration in maize.

Implications of the Results on Breeding for ProVA

Drought-stress tolerance and nitrogen-use efficiency are important breeding objectives for maize breeding programs in sub-Saharan Africa (Bänziger et al., 2000; Bänziger et al., 2006; Cairns et al., 2013). The results of this study suggested that when improving maize for carotenoid content, breeders could use the same germplasm for optimum, drought stress, or low-N conditions, as long as it has sufficient genetic variance for grain yield and other relevant traits. The consistently high contribution of GCA to total sums of squares under optimum conditions, low-N, and drought stress suggests that breeding methods that exploit additive genetic variance will be effective in developing high proVA OPCs or hybrids. Rapid increase in population means for proVA carotenoids has been demonstrated by Dhliwayo et al. (2014), who reported >25% linear increase per cycle from S_1 recurrent selection for proVA carotenoids in three populations. Similarly, Pixley et al. (2013) showed that proVA content increased from 2 to 3 mg g^{-1} in normal yellow maize in 2004 to 7 to 8 mg g^{-1} in the first released biofortified hybrids in 2012, an improvement of about 300% in 8 yr.

Negligible variations in the magnitude of phenotypic correlation coefficients among traits across stress treatments (Table 6) suggested that the genetic relationship among carotenoids was not altered by either drought stress or low-N stress. This result further confirmed our general finding that although carotenoid content might be affected by stress, the G × E interaction was negligible. These results suggested that the breeding strategies and methods used to develop proVA hybrids and OPCs under optimum conditions may

be effective for drought-prone and for farming systems where nitrogen fertilizer is a limiting factor.

Selecting for the favorable allele at the *CRTRB1* locus increases β-carotene at the expense of β-cryptoxanthin. In hindsight, selecting for the partial loss-of-function mutation of *CRTRB1* to increase proVA seems to have been a strategic mistake, considering increasing evidence that shows β-cryptoxanthin to be equally or more bioavailable (Howe and Tanumihardjo, 2006; Schmaelzle et al., 2013; Sugiura et al., 2014) and less prone to degradation (Taleon et al., 2017) than β-carotene. A long-term breeding strategy that relies less on reducing the hydroxylation function of *CRTRB1* to increase both β-carotene and β-cryptoxanthin may be more appropriate. The high heritability estimates reported for proVA carotenoids and the well-characterized carotenoid biosynthetic pathway make it amenable to MAS. To simultaneously improve grain yield and proVA carotenoids, a breeding strategy that combines independent culling for proVA with index selection for grain yield and other agronomic traits may be suitable. Such a strategy could involve rapid-cycle genomic selection (Owens et al., 2014) using known genes associated with the carotenoid biosynthesis pathway as fixed factors, whereas index selection could be performed based on predictions for other target environments, including optimum, drought-stress, and low-N (Bernardo, 2014; Cerrudo et al., 2018).

CONCLUSIONS

Results obtained in this study provide insights that may help breeders to design effective breeding strategies to develop provitamin A-enriched cultivars for resource-limited farming systems. We show that provitamin A carotenoids were generally reduced under drought stress and low-N conditions, relative to optimum conditions. Carotenoids were primarily controlled by strong additive genetic effects under drought stress, low-N, and optimum conditions. Negligible genotype × environment interaction suggested that cultivars with high provitamin A content under optimum conditions can be grown under drought stress and low-N conditions, as long as they are sufficiently adapted for grain yield and other agronomic traits. The weak genotype × environment interaction for carotenoids also suggests that the same germplasm and breeding strategy may be used across a broad range of maize production conditions. A significant negative correlation was detected between grain yield and provitamin A carotenoids, but it was deemed an artifact of the genetic background of the lines, rather than tight linkage of loci controlling the two traits, or pleiotropy. Additional studies using more diverse inbred lines than those used in this study may be needed to confirm our findings.

Conflict of Interest

The authors declare that there is no conflict of interest.

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