

Combining ability, maternal and reciprocal effects for growth traits, total carotenoids and beta carotene in yellow maize (*Zea mays* L.)

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Submitted:
30/09/2025

Revised:
17/11/2025

Accepted:
27/12/2025

Abstract: Vitamin A deficiency (VAD) is a major public health problem in Ghana where almost 95% of the maize produced is consumed by humans. Despite efforts to improve the crop, most PVA varieties have not met the current international target of combating VAD. To improve the beta carotene content of yellow maize it expedient to understand the nature of gene action driving the performance and heritability of the trait to identify and select best performing genotypes as parents for further breeding aimed at improving the beta carotene content. Two genotypes NZER1 (local landrace) and HONAMPA (PVA variety) and four PVA genotypes (DZIFO, AHOOF, LY1203-20, LY1501-6) were planted and crossed on the field using Griffins diallel method I model I. The study revealed significant variation for total carotenoids and beta-carotene content ranging between 4.947 µg/g to 19.19 µg/g with a mean of 10.91 µg/g and 1.096 µg/g to 5 µg/g with a mean of 2.062 µg/g respectively. AHOOF and DZIFO revealed high GCA effects for beta carotene content as male parents and DZIFO and LY 15 had high GCA effects as female parents and therefore could be considered for progeny selection. High SCA effects were obtained by HON*NZE (3.516), HON*LY12 (3.333), HON*LY15 (3.442), AHO*DZI (4.224), AHO*NZE (2.465) for beta carotene. AHO*DZI, HON*LY15, DZI*LY12 had high SCA effect for total carotenoid. These parental combinations will best useful for development of hybrid varieties with improved beta carotene content.

Key words: Breeding, combining ability, maternal effects, deficiency, bio-fortification.

Introduction

Maize is a very important crop in the world economy and a major staple for many people in the developing world including sub Saharan Africa, where it is a major staple for an estimated 50% of the population (Badu-Apraku et al., 2010). Maize is consumed by more than a billion people in sub-Saharan Africa, Latin America and in many countries in Asia, (Gupta et al., 2009, Shiferaw et al., 2011). Ghana produced 3255718 tons in 2022 (FAOSTATS, 2024) most of which is consumed domestically.

Yellow maize rich in beta carotene might be recommended as an efficient food source to combat Vitamin A deficiency (VAD) in countries where maize is already a staple food and where VAD is a public health problem (Muzhingi et al., 2011). VAD is a major public health problem in most developing countries including Ghana where almost 95% of the maize produced are consumed by humans (Halilu et al., 2016), VAD is responsible for a number of disorders including impaired iron mobilization, growth retardation, blindness, depressed immune response, increased susceptibility to infectious disease and increased childhood mortality (Sommer and Davidson, 2002; WHO, 2009). Reducing vitamin A deficiency in developing countries through bio-fortification of staple foods has higher potential than food fortification methods that are expensive, unsustainable and not easily accessible to people in rural communities (Muzhingi et al., 2011, Sommer and Davidson, 2002). Yellow maize contains considerable levels of β-carotene (Menkir et al., 2008, Safawo et al, 2010) and has high natural variation for carotenoids (Mishra et al., 2010). Typical yellow maize varieties have 0.5 to 1.5 µg/g pro-vitamin A (PVA) (Egesel et al., 2003, Safawo et al., 2010) which is inadequate to prevent VAD in populations using maize dominated diets,

the current international target set by harvest plus (www.harvestplus.org) for combating VAD using maize is the development of maize kernels with beta carotene content of 15 µg/g (Harjes et al., 2008, Halilu et al., 2016).

Despite efforts to improve the crop, most PVA varieties have not met the current international target of combating VAD (Harjes et al., 2008, Halilu et al., 2016). In order to improve the beta carotene content of yellow maize it expedient to understand the nature of gene action driving the performance and heritability of the trait, select the best breeding method for improving this trait and also identify best performing genotypes as parents for further breeding aimed at improving the beta carotene content towards the target of 15 µg/g.

Diallel analysis has been extensively used in both self and cross-pollinated species to understand the nature of gene action involved in the expression of quantitative traits (Singh, 2015, Singh et al., 2015) and some qualitative trait (Jindal et al., 2015). This is based on the estimation of general combining ability (GCA) and specific combining ability (SCA) variances and effects. Sprague and Tatum defined GCA as the average performance of a genotype in a series of hybrid combinations. Ali et al., 2014a; Ali et al., 2013, Masood et al., 2015a defined SCA as those cases in which certain hybrid combinations perform better or poorer than would be expected on the basis of the average performance of the parental lines. The objective of this project is to estimate the total carotenoids and beta carotene content of parents and F₁'s, provide information about GCA and SCA effects, estimate maternal effects, reciprocal effects and to understand the nature of gene action involved in the expression of maturity, carotenoids and beta carotene traits. Also to identify and select suitable parents, superior cross combinations and the appropriate breeding procedure for further breeding purposes aimed at development new yellow maize varieties with improve beta carotene content.

Results

Days to 50% anthesis, days to 50% silking, anthesis silking interval, days to 50% leaf senescence

All the genotypes were found to be medium maturing with a mean days to 50% leaf senescence of 91.805 days and a range of between 84 to 99 days, days to 50% anthesis varied between 52 and 66 days with a mean of 58.638 days, the mean days to 50% silking was 63.22 days but varied between 54 and 70 days. Anthesis silking interval varied between 2 and 8 days with a mean of 4.5 days.

Variability in beta carotene and total carotenoid content

The mean beta carotene content was 2.061 µg/g with a minimum of 1.096 and a maximum of 5 µg/g (Fig. 2), however total carotenoid content varied between 10.112 µg/g and 19.907µg/g (Fig. 3).

Correlation analysis

Significant high correlation was found between days to 50% anthesis, 50% silking and days to 50% leaf senescence ($r=0.96$, $r=0.99$ respectively) and moderately negatively correlated with anthesis silking interval but low and positively correlated with beta carotene and total carotenoid content. Significant positive correlation was found between total carotenoid and beta carotene content ($r=0.66$) (Table 1).

Estimation of general combining ability and maternal effects

High positive GCA effects were recorded by AHOOFE (1.13) and NZER1 (2.53) with NZER1 revealing high maternal effect (2.4) whilst HONAMPA recorded a high negative GCA effect (-3.06) as male parent. LY 12 (3.13) and DZIFO (1.53) recorded high positive GCA effects with LY 15 (1.00) recording high maternal effects for days to 50% anthesis AHOOFE and NZER1 recorded high positive GCA effects (1.27, 2.47) as male parents and DZIFO and LY12 recorded high GCA as female parents with NZER1 recording high maternal effect (2.1) for days to 50% silking (table 3).

High GCA effects were recorded by HONAMPA as male parent (0.73), DZIFO, NZER1, and LY 15 as female parents. HONAMPA and AHOOFE (0.9, 0.3 respectively) had high maternal effect for anthesis silking interval. NZER1 had high GCA effect (2.37) and maternal effect 2.3 for days to 50% leaf senescence (Table 4).

High GCA effects were revealed by AHOOFE (0.98), DZIFO (0.22) as male parents. AHOOFE also revealed high maternal effects (4.12). DZIFO (0.21) and LY15 (0.22) exhibited high GCA effects as female parents. With respect to the total carotenoid content NZER1 (2.21) and DZIFO (1.23) had high GCA effect as male parents and AHOOFE was the best combiner as female parent with a high GCA effect (4.12). The study revealed high maternal effects in total carotenoid content for DZIFO and NZER1 (1.91 and 1.76 respectively). A high maternal effect was recorded for beta carotene content in AHOOFE (0.72) (table 5).

Estimates of SCA effects and reciprocal effects

The highest SCA effect was recorded by DZI×LY15 (10.27) followed by NZE×LY15 (6.53), AHO×LY12 (6.47), HON×NZE (5.07), AHO×DZI (4.67), AHO×NZE (4.27) and AHO×LY15 (4.13) for days to 50% anthesis. The following F₁'s had high reciprocal effects for days to 50% anthesis. AHO×DZI (6.20), DZI×LY15 (5.90), HON×LY12 (2.49) and DZI×NZE (2.30) all the remaining genotypes had low reciprocal effects. High SCA effects were recorded by DZI×LY15 (9.35), NZE×LY15 (5.67) and HON×NZE (5.53) for days to 50% silking. DZI×LY15, AHO×DZI and DZI×NZE had high reciprocal effects for days to 50% silking all the other F₁'s had low reciprocal effects (table 5).

Low SCA and reciprocal effects was recorded for all the F₁'s for anthesis silking interval. The SCA effect for days to 50% leaf senescence was found to be low in most crosses except DZI×LY15 (10.433), AHO×LY12 (6.43), HON×NZE (5.23),

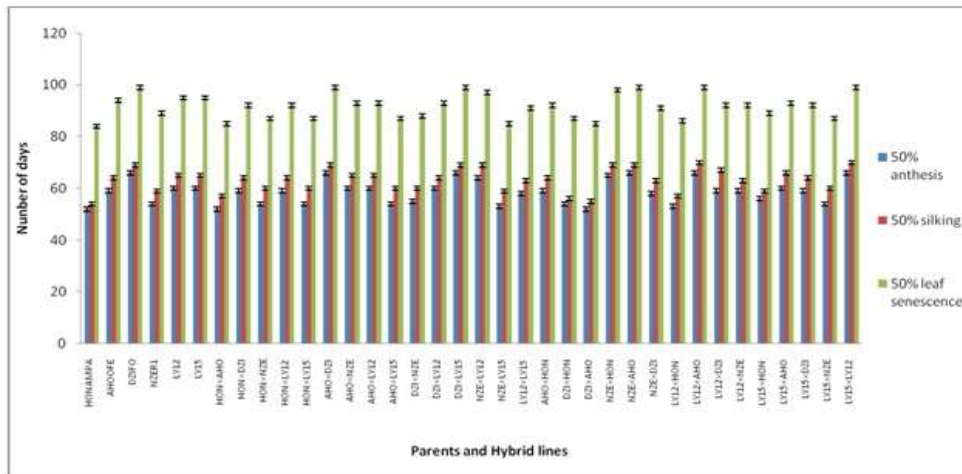


Figure 1. Days to 50% anthesis, days to 50% silking, anthesis silking interval, Days to 50% leaf senescence.

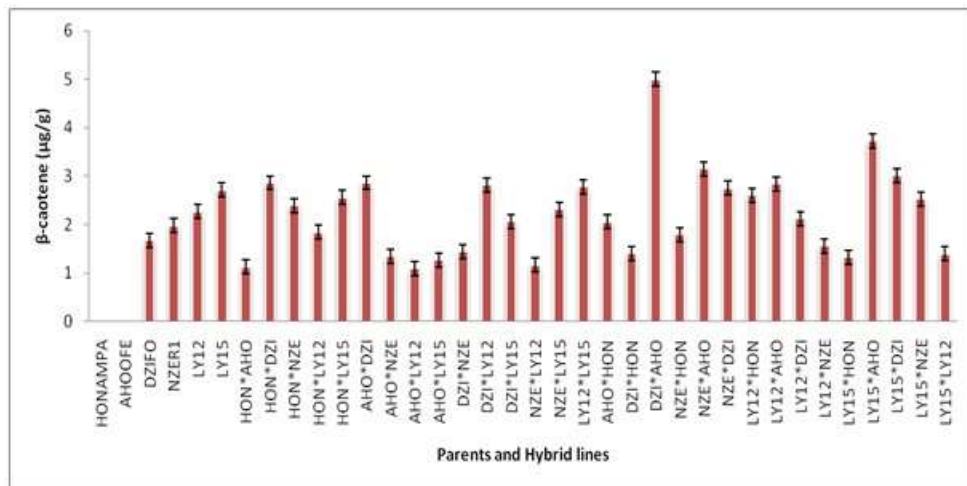


Figure 2. Variability in beta carotene content.

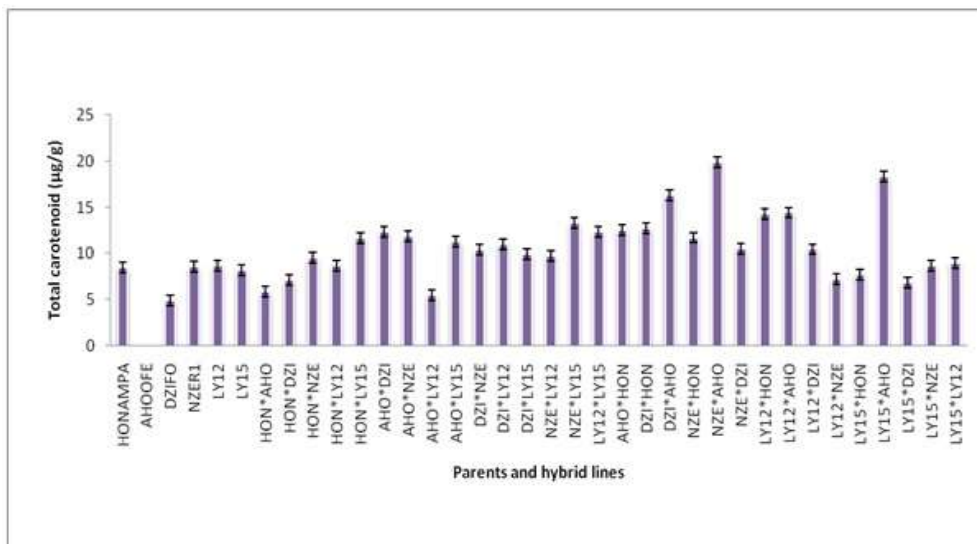


Figure 3. Variability in total carotenoid content.

AHO×DZI (4.63) and AHO×NZE (4.43) which recorded high SCA effects. DZI×LY15 (6.00), AHO×DZI (5.30) and HON×LY12 (2.50) revealed high reciprocal effects (table 6).

Low positive SCA effects were revealed by AHO× HON (0.67), HON×LY15 (0.71), AHO×DZI (0.92), DZI×AHO (1.78) and DZI×LY12 (0.8) for total carotenoid content. Reciprocal effects were also low, LY15×HON, DZILY12 and LY12×LY15 obtaining 0.72, 0.64 and 0.71 respectively for total carotenoid. However, with the beta carotene content AHO×NZE (8.17), LY15×AHO (10.27), NZE×HON (3.52), LY15×HON (3.44) and DZI×AHO (4.22) recorded the high positive SCA effects whilst LY12×DZI (-5.41) and LY15×DZI (-4.12) recorded high negative SCA effects. High reciprocal effects were revealed by LY15×HON (2.27), DZI×AHO (2.05) and LY12×DZI (3.19) for beta carotene (Table 7).

Table 1. Correlation analysis.

	50% anthesis	50% leaf senescence	50% silking	ASI	Bc	tc
50% anthesis	-					
50% leaf senescence	0.99	-				
%50% silking	0.96	0.96	-			
ASI	-0.22	-0.22	0.06	-		
bc	0.04	0.02	0.06	0.06	-	
tc	0.04	0.01	0.01	-0.09	0.66	-

50% anthesis= days to 50% anthesis, 50% leaf senescence=days to 50% leaf senescence, 50% silking= days to 50% silking, ASI= anthesis silking interval, bc= beta carotene content, tc= total carotenoid content.

Table 2. Estimates of GCA effects for male and female parents and maternal effect days to 50% anthesis, days to 50% silking.

Parent	♂GCA ant	♀GCA ant	M eff	♂GCA sil	♀GCA sil	M eff
HONAMPA	-3.06	-0.26	-1.40	-2.33	-2.33	0.00
AHOOFE	1.13	0.53	0.30	1.27	0.07	0.60
DZIFO	-1.26	1.53	-1.40	-2.53	2.07	-2.30
NZER1	2.53	-2.26	2.40	2.47	-1.73	2.10
LY12	0.33	3.13	-1.40	0.67	3.07	-1.20
L715	0.33	-1.66	1.00	0.47	-1.13	0.80

ant= days to 50% anthesis, sil= days to 50% silking, M eff= maternal effect.

Table 3. Estimates of GCA effects for male and female parents and maternal effect anthesis silking interval and days to 50% senescence.

Parent	♂GCA ASI	♀GCA ASI	M eff	♂GCA dts	♀GCA dts	M eff
HONAMPA	0.73	-1.06	0.90	-3.03	-1.23	-0.90
AHOOFE	0.13	-0.46	0.30	1.17	0.57	0.30
DZIFO	-1.26	0.53	-0.90	-1.23	1.57	-1.40
NZER1	-0.06	0.53	-0.30	2.37	-2.23	2.30
LY12	0.33	-0.06	0.20	0.37	3.16	-1.39
L715	0.13	0.53	-0.20	0.37	-1.83	1.10

Ls=days to 50% leaf senescence, ASI= anthesis silking interval, M eff= maternal effect.

Discussion

All the F1's under study were medium maturing except HON×AHO, DZI×AHO and NZE×LY15 which matured relatively earlier than the others. Early maturity is an important trait considered by farmers when selecting their preferred maize varieties. Medium maturing maize varieties tend to yield more than early maturing ones and yield is the most important farmer preferred trait, therefore medium maturing varieties that tend to be a balance between yield and maturity period is most preferred by farmers. The study revealed significant variation for total carotenoids, similar results was observed by Harjes et al., 2008 and Rashmi et al. (2014). Similarly, significant variation for kernel carotenoids in established maize inbred lines has also been reported by Mishra et al. (2010) and Tiwari et al. (2012) The present study revealed significant variation for beta-carotene content similar to Egessel et al. (2003); Rashmi et al. (2014) who reported beta-carotene concentration of 0.5µg/g to 3.4µg/g with mean value of 1.5µg/g and 4.814 µg/g with a mean of 2.58µg/g respectively, when working on maize hybrids. A range of 0.7 µg/g to 4.7µg/g was recorded across four trials with mean of 1.9µg/g of kernel beta-carotene was reported by Menkir et al. (2008) while evaluating a group of tropical yellow maize inbreds. Total carotenoids also recorded significant high correlation with beta carotene content. This indicates that selecting for any of this trait will mean selecting for the other. The high GCA effect recorded by NZER1, DZIFO and LY12 for days to 50% anthesis and days to 50% silking indicates that additive genes control these traits and therefore progeny selection is the best method recommended for improving these traits. NZER1 has high GCA effect and maternal effect for days to 50% leaf senescence. AHOOFE and DZIFO revealed high GCA effects for beta carotene content as male parents and DZIFO and LY 15 were found to best combiners as female parents and therefore could be considered for progeny selection.

High SCA effects were obtained by HON×NZE, HON×LY12, HON×LY15, AHO×DZI, AHO×NZE for beta carotene. NZE×AHO and LY15×AHO recorded high SCA effects. According to Sprague and Tatum 1942, Deitos 2006 and Machikowa 2011 the SCA is controlled by non-additive gene action. High SCA effects resulting from crosses where both parents are good general combiners (i.e., good GCA × good GCA) maybe ascribed to additive × additive gene action (Chigeza et al., 2014; Dey et al., 2014). The high SCA effects derived from crosses including good × poor general combiner parents may be attributed to

Table 4. Estimates of GCA effects for male and female parents and maternal effect for beta carotene, total carotenoids.

Parent	♂GCA bc	♀GCA bc	M eff	♂GCA tc	♀GCA tc	M eff
HONAMPA	-0.34	-0.35	0.01	-3.48	0.96	-2.22
AHOOFE	0.98	-0.46	0.72	-0.13	4.12	-2.12
DZIFO	0.22	0.36	-0.07	1.23	-2.58	1.91
NZER1	-0.33	0.05	-0.19	2.20	-1.31	1.76
LY12	-0.53	0.19	-0.36	0.90	-2.06	1.48
LY15	0.01	0.22	-0.11	0.74	0.87	-0.06

bc= beta carotene content, tc= total carotenoid content, M eff= maternal effect.

Table 5. Estimates of SCA effects and reciprocal effects for days to 50% anthesis, days to 50% silking.

Direct crosses	Reciprocal crosses	SCA 50% anthesis direct crosses	SCA reciprocal crosses	reciprocal effects	SCA 50% silking direct crosses	SCA reciprocal crosses	reciprocal effects
HON×AHO	AHO×HON	-4.13	0.47	-2.30	-4.07	1.73	-2.90
HON×DZI	DZI×HON	1.87	-2.13	1.99	0.93	-2.45	1.69
HON×NZE	NZE×HON	0.67	5.07	-2.20	0.73	5.53	-2.40
HON×LY12	LY12×HON	0.27	-4.73	2.49	-0.07	-4.67	2.30
HON×LY15	LY15×HON	0.07	-1.73	0.89	0.13	-2.47	1.30
AHO×DZI	DZI×AHO	4.67	-7.73	6.20	2.33	-5.85	4.09
AHO×NZE	NZE×AHO	2.47	4.27	-0.90	2.13	3.13	-0.50
AHO×LY12	LY12×AHO	-2.93	6.46	-4.70	-2.67	5.93	-4.30
AHO×LY15	LY15×AHO	-4.13	0.46	-2.30	-3.47	2.13	-2.80
DZI×NZE	NZE×DZI	-0.13	-4.73	2.30	0.95	-4.87	2.91
DZI×LY12	LY12×DZI	-0.53	-1.53	0.50	0.15	0.93	-0.39
DZI×LY15	LY15×DZI	10.26	-1.53	5.90	9.35	-1.87	5.61
NZE×LY12	LY12×NZE	-0.33	2.27	-1.30	0.13	0.73	-0.30
NZE×LY15	LY15×NZE	-6.53	-2.73	-1.90	-5.67	-2.07	-1.80
LY12×LY15	LY15×LY12	0.67	3.87	-1.60	0.13	3.13	-1.50

50% anthesis= days to 50% anthesis, 50% silking= days to 50% silking.

Table 6. Estimates of SCA and reciprocal effects for Anthesis-Silking Interval and Days to 50% leaf senescence.

Direct crosses	Reciprocal crosses	SCA ASI direct crosses	SCA Reciprocal crosses	reciprocal effects	SCA 50% leaf senescence direct crosses	SCA reciprocal crosses	reciprocal effects
HON×AHO	AHO×HON	0.07	1.27	-0.60	-4.17	0.43	-4.80
HON×DZI	DZI×HON	-0.93	-0.33	-0.30	1.83	-2.17	2.00
HON×NZE	NZE×HON	0.07	0.47	-0.20	0.63	5.23	-2.30
HON×LY12	LY12×HON	-0.33	0.07	-0.20	0.23	-4.77	2.50
HON×LY15	LY15×HON	0.07	-0.73	0.40	0.23	-1.77	1.00
AHO×DZI	DZI×AHO	-2.33	0.07	-1.20	4.63	-5.97	5.30
AHO×NZE	NZE×AHO	-0.33	-1.13	0.40	2.43	4.43	-1.00
AHO×LY12	LY12×AHO	0.27	-0.53	0.40	-2.97	6.43	-4.70
AHO×LY15	LY15×AHO	0.67	1.67	-0.50	-3.97	0.43	-2.20
DZI×NZE	NZE×DZI	-0.13	-0.13	0.00	-4.57	-0.17	-2.20
DZI×LY12	LY12×DZI	0.67	2.47	-0.90	-0.57	-1.57	0.50
DZI×LY15	LY15×DZI	-0.93	-0.33	-0.30	10.43	-1.57	6.00
NZE×LY12	LY12×NZE	0.07	-1.53	0.80	-0.17	2.23	-1.20
NZE×LY15	LY15×NZE	0.47	0.67	-0.10	-7.17	-2.77	-2.20
LY12×LY15	LY15×LY12	-0.33	-0.73	0.20	0.83	3.83	-1.50

dts = days to 50% leaf senescence, ASI = Anthesis Silking Interval.

Table 7. Estimation of SCA effects and reciprocal effects for total carotenoid and beta carotene content.

Direct crosses	Reciprocal crosses	SCA tc direct crosses	SCA Reciprocal crosses	Reciprocal effects	SCA bc direct crosses	SCA Reciprocal crosses	Reciprocal effects
HON×AHO	AHO×HON	-1.69	0.67	-1.18	-5.63	0.88	-3.26
HON×DZI	DZI×HON	-0.76	-0.49	-0.13	-3.69	-0.28	-1.70
HON×NZE	NZE×HON	0.88	-0.09	0.49	3.52	1.25	1.13
HON×LY12	LY12×HON	0.52	0.57	-0.02	3.33	1.70	0.81
HON×LY15	LY15×HON	0.71	-0.74	0.72	3.44	-1.10	2.27
AHO×DZI	DZI×AHO	0.92	1.78	-0.42	4.22	0.13	2.05
AHO×NZE	NZE×AHO	-0.04	-0.06	0.01	2.46	8.17	-2.85
AHO×LY12	LY12×AHO	-0.10	-0.52	0.20	-3.16	1.71	-2.44
AHO×LY15	LY15×AHO	-0.47	0.34	-0.40	-0.27	10.27	-5.27
DZI×NZE	NZE×DZI	-0.77	0.29	-0.53	-0.39	-0.45	0.03
DZI×LY12	LY12×DZI	0.80	-0.48	0.64	0.98	-5.41	3.19
DZI×LY15	LY15×DZI	-0.49	0.38	-0.44	-3.02	-4.12	0.55
NZE×LY12	LY12×NZE	-0.54	-0.49	-0.03	-1.21	-1.98	0.38
NZE×LY15	LY15×NZE	0.07	0.45	-0.19	-0.61	-0.14	-0.24
LY12×LY15	LY15×LY12	0.39	-1.02	0.71	-0.27	0.97	-0.62

tc=total carotenoids, bc= beta carotene content.

favorable additive effects of the good general combiner parent and epistatic effects of poor general combiner, which fulfils the favorable plant attribute. High SCA effects manifested by low × low crosses can lead to over dominance (Chigeza et al., 2014, Dey et al., 2012). AHO×DZI, HON×LY15, DZI×LY12 had high SCA effect for total carotenoid, thus hybrid development will be an appropriate breeding method for the development of maize varieties with high pro-vitamin A contents adaptable to the tropics (Halilu et al., 2016). High maternal effect was revealed by AHO×FE and hence its performance as a good combiner as female parents indicating the involvement of cytoplasmic genes in the expression of the beta carotene trait. AHO×DZI and HON×AHO recorded high reciprocal effects for total carotenoids

Materials and Methods

Experimental site

The experiment was carried out at the Noguchi Memorial Institute for Medical Research in January 2022 after maize has been planted and grown to maturity and dried at the Biotechnology and Nuclear Agriculture Research Institute of the Ghana Atomic Energy Commission in June 2021

Source of germplasm

Two genotypes NZER1 (local landrace) and HONAMPA (existing PVA variety) were selected from 100 local accessions characterized in a previous experiment based on their high beta carotene content (Ansah et al., 2023) and four PVA genotypes (DZIFO, AHO×FE, LY1203-20, LY1501-6) obtained from the Crop Research Institute were planted and mated on the field using Griffins diallel method I model I (Griffing, 1956) where each parent has the opportunity to mate with any other parent thereby obtaining direct crosses and reciprocal crosses generating thirty F₁s. The F₁s and parents were evaluated on the field by planting on 18th June 2021 during the major season using 6×6 lattice design with three replications at the same location.

Carotenoids and Beta-carotene quantification using HPLC

Beta-carotene extraction was prepared according to a protocol described by Rodriguez-Amaya and Kimura, (1998). Part of the extract was used for High Pressure Liquid Chromatography (HPLC) and another part for the measurement of absorbance. Agilent HPLC 12 equipment was employed for the quantification of the beta-carotene content. In brief, 250 ml of prepared extract was evaporated under steam of nitrogen gas and then reconstituted with 50 µl of the mobile phase made of hexane and benzene. 20 µl of the reconstituted sample was injected into the HPLC machine and the readings were recorded.

Data analysis

Several concentrations of the standard were used to develop a calibration curve, the linear relationship was found and model was used to calculate the beta carotene content by placing the area under the curve in the model. Total carotenoids were calculated using the formula proposed by Rodriguez Amaya and Kimura (1998)

The absorbance coefficient for beta carotene is 2592.

Means were separated by error bars with standard error

Griffing Method 1 MODEL 1 was used to estimate combining ability (Griffing, 1956). The analysis was based on the model by Griffing as:

$$X_{ij} = \mu + g_i + g_j + s_{ij}$$

where X_{ij} = the mean phenotypic value; μ = the general mean; g_i, g_j = GCA effects of the i th and j th parents, respectively; s_{ij} = SCA effect of the cross $i \times j$.

The estimates of GCA and SCA of parents and hybrids were obtained as:

GCA effects $g_i = X_{ij...k} - \mu$

SCA effects $s_{ij} = X_{ij} - \mu - g_i - g_j$

Where X_i, X_j = means of the i th and j th parents, respectively; X = grand mean; $X_{ij...k}$ = mean of parental performance

Maternal effect was calculated according to Cockerham (1963) as average difference between m_{gi} and f_{gi} .

Reciprocal effect was calculated as the difference between SCA of direct crosses and SCA of reciprocal crosses (Ilyas, 2007)

Conclusion

Maternal and reciprocal effects can be used to predict the performance of selected parents and also select the best breeding method for future breeding of high yielding varieties with improved carotenoid and beta carotene contents. AHOFE, DZIFO and LY12 which recorded high GCA and maternal effects could be selected for progeny selection, HON×NZE, HON×LY12, HON×LY15, AHO×DZI, AHO×NZE which had high SCA effects can be selected for hybrid breeding. These parental combinations will be best useful for development of hybrid varieties with improved beta carotene content for elimination of vitamin A deficiency and promotion of food security.

Acknowledgements

The authors wish to acknowledge the contribution of Dr. Francis Egbi and Madam Evelyn Boakye Danquah of the Noguchi Memorial Institute for Medical Research during the beta carotene extraction and quantification.

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