



Full Length Article

Evaluation of Single Cross Yellow Maize Hybrids for Agronomic and Carotenoid Traits

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Abstract

Provitamin A biofortification of maize endosperm was found to be most convenient solution to the resolve its deficiency in the masses. Continuous efforts for persistent evaluation of diverse genetic material are prerequisite for genetic improvement of maize endosperm for provitamin A. Distinctively selected inbred lines and testers were evaluated for their yield and provitamin A related performance in single cross F₁ hybrid combinations. Total 50 single cross hybrids were evaluated for different agronomic and carotenoid related traits. Significant differences were observed among genotypes, lines, testers and line × tester interaction for studied traits. L5, L6 and T1 had high GCA effects for YPH, RPC and FW whereas, L1, L5 and T4 had high GCA effects for TCC, PVAC and NPVAC. Among studied crosses, L7×T3, L8×T4 and L5×T2 had high SCA effects for PVAC whereas, L2×T5, L3×T3 and L4×T2 had high SCA effects for YPH. L3×T3 had high SCA effects for YPH, TCC, PVAC and NPVAC. L8×T4 had high SCA effects for PVAC, RPC and FW. L2×T5 had high SCA effects for PVAC, YPH, NPVAC and TCC. Most of variability was contributed by L×T interaction which showed that performance of the parents could only be evaluated in specific cross combinations. Phenotypic variances and phenotypic coefficient of variations were higher which showed that performance of the crosses was also dependent on the environmental factors. Gene action showed that all of the traits were under the control of non-additive gene action which strongly directs the manipulation of heterosis breeding for genetic improvement of studied traits. L3×T3, L5×T4, L3×T1, L4×T3, L2×T1, L8×T3 and L5×T2 for RPC and TCC whereas, L1×T1, L3×T5, L7×T1, L10×T5, L7×T2 and L9×T1 for FW, YPH and PVAC had highest better parent heterosis. Results of Kempthorne's analysis and GGE biplot analysis were comparable for estimation of GCA, SCA effects and identification of desired crosses. Therefore, GGE biplot could be preferred for providing visual explanation of the effects and additionally provides information about heterotic grouping of testers, SCA effects of parents, best parent and best crosses. Combining ability, gene action and heterosis studies based on Kempthorne's analysis and GGE biplot analysis revealed that genetic improvement can be made for studied agronomic and carotenoid related traits. Improvement of yield and provitamin A carotenoids may help the peoples of Pakistan to suffice the food security issue and to alleviate the sub-clinical symptoms of vitamin A deficiency. © 2017 Friends Science Publishers

Keywords: GCA effects; SCA effects; Heterosis; Gene action; Best parent; Line × tester analysis; GGE biplot analysis

Introduction

Vitamin A deficiency (VAD) is responsible for wide range of mortality, morbidity, night blindness, xerophthalmia, problems of tears glands, scaly or toad like skin, immune disorders and vulnerability to infectious diseases (World Health Organization, 2004, 2009; HarvestPlus, 2008; Sherwin *et al.*, 2012). Almost 190 million preschool children and 20 million females at pregnancy or lactating stages are having the serious deficiency of vitamin A in many developing countries of Africa and Asia (Darnton-Hill *et al.*, 2005; The World Bank, 2006). Due to VAD, mortality rate is higher in Pakistan than India, Nepal and

Bangladesh. Pakistan also has severe sub-clinical deficiency of vitamin A in masses (World Health Organization 2004). Pakistan Institute of Community Ophthalmology (2013) reported that exclusively large number of children below the age 15 years are seriously facing the problem of xerophthalmia. About 61% peoples (among them 36% were females and 64% were males) were reported to have blinding xerophthalmia, corneal ulcer, keratomalacia and corneal xerosis in Pakistan. Prevalence of VAD is also increasing in Pakistan since 2001 because at that time only 5% females were victim of VAD whereas, currently in 2013 about 42% females were facing the same problem (Balagamwala and Gazdar, 2013). Therefore, there is dire

need to combat this problem of hidden hunger in Pakistan to improve the health status of the peoples. World Health Organization (2009) declared multiple interventions like, diversifying the dietary foods to increase the vitamin A intake, supplementation through vaccines or capsules, industrial fortification of dietary food items and biofortification of food crops to overcome the prevailing problem of vitamin A deficiency. Crop biofortification is prescribed as most convenient solution to combat the malnutrition due to DAILYs (disability adjusted life years), affordability, accessibility, acceptability and being economical to intervene.

Vitamin A from the plant sources is called provitamin A carotenoids those are comprised of α -carotene, β -carotene and β -cryptoxanthin. These provitamin A carotenoids are commonly present in different fruits, vegetables, legumes and cereal crops. Among these crops, maize is most appropriate to target for provitamin A biofortification due to higher potential genetic variability for provitamin A carotenoids, higher bioavailability, lower bioconversion ratio and easy to incorporate in daily diet. Variability in the carotenoid contents of maize kernels indicate that concerned metabolic pathways could be absent, inhibited or truncated in the endosperm (Naqvi *et al.*, 2009). Harjes *et al.* (2008) and Yan *et al.* (2010) contributed significantly to exploit the genetic variability in yellow maize endosperm for provitamin A carotenoids. To alleviate the issue of VAD in Pakistan through biofortification of maize, necessitates the exploitation of heterotic potential of maize. To date, no attempt was reported in the literature to exploit the indigenous maize germplasm for heterotic potential of provitamin A carotenoids.

Different mating designs including diallele mating design, line \times tester mating and biparental crosses are used for evaluation of heterotic potential and combining abilities of maize hybrids. Line \times tester analysis which is modified version of top cross design is most commonly used for understanding the combining abilities of parents and crosses along with the estimation of hereditary pattern of targeted traits (Sharma, 2006). This method is advantageous over the other methods because evaluation could easily be made with lesser experimental material. This mating design has been extensively used for studying the agronomic traits, yield components and quality traits (Bekele and Rao, 2013; Mahesh *et al.*, 2013; Amin *et al.*, 2014). Different mating designs have wide range of applicability but there is basic and common problem associated with them is the visual presentation of the results. For improving the comprehensibility and visualization of the results from line \times tester analysis based studies, different alternative methods are being explored. GGE biplot analysis provides best graphical visualization of the results for two way data tables (Badu-Apraku *et al.*, 2013; Ruswandi *et al.*, 2015). Thus, GGE biplot could be used as graphical approach for combining ability estimation of line \times tester data and to decide that whether it could be used as alternative to the

conventional Kempthorne's numerical approach. Thus key objectives of this study were to evaluate the diverse yellow maize inbred lines for combining ability and heterotic potential by using conventional Kempthorne's numerical approach and GGE biplot analysis for provitamin A carotenoids and yield related traits. Assessment of gene action for provitamin A contents and yield related traits in indigenous genetic material was also key objective of this study.

Materials and Methods

Plant Material

Yellow maize germplasm comprising of 150 genotypes was collected from Plant Genetic Resource Institute (PGRI), National Agricultural Research Council (NARC), Islamabad, Pakistan. These genotypes belong to different agro-ecological regions of Pakistan according to passport information provided by PGRI. These 150 genotypes were evaluated for provitamin A contents and yield potential for two consecutive seasons (spring and autumn 2015). After the evaluation of these homogenous 150 genotypes, 10 genotypes were selected as inbred lines and 5 genotypes were selected as testers based on grain yield and provitamin A contents. These selected parents (10 lines and 5 testers) were crossed by using line \times tester mating (LTM) design in research area of Department of Plant Breeding and Genetics, University of Agriculture Faisalabad which is located at 73.1° longitude, 31.43° latitude and 184.5 m altitude. Names of these selected parents were coded as L1: 15068, L2: 15076, L3: 15071, L4: 15163, L5: 24687, L6: 14959, L7: 14970, L8: 15205, L9: 24689, L10: 15350, T1: 15055, T2: 15052, T3: 15073, T4: 15130 and T5: 14966. Numerical coding used for these genotypes in present study is according to the passport information of PGRI, NARC. Temperature and precipitation conditions of experimental site during development of single crossed F₁ hybrids during spring 2016 and evaluation of single crossed F₁ hybrids during autumn 2016 were given in Fig. (1).

Field Evaluation

Fifty single cross yellow maize hybrids and parents were evaluated in the field during autumn season 2016. These F₁ hybrids were planted on two rows of five meter length with 0.75 m between rows and 0.20 m within rows distance. Standard agronomic practices were followed during hybrid development (spring, 2016) and hybrid evaluation (autumn, 2016) studies. These fifty F₁ single crosses were evaluated under randomized complete block design with three replications of each cross. Data were collected for plant height (PH; cm), ear height (EH; cm), days to 50% silking (DS), days to 50% tesseling (ST), anthesis silking interval (ASI), grains per row (GPR), rows per cob (RPC), field weight (FW; kg), yield per hectare (YPH; tons ha⁻¹),

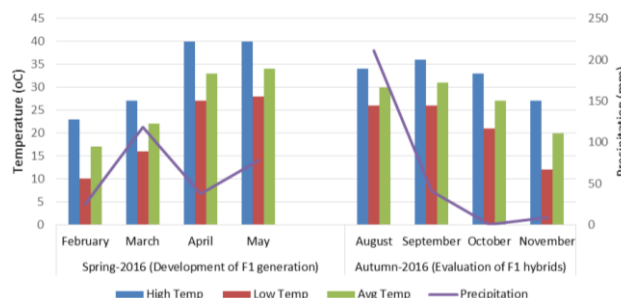


Fig. 1: Meteorological conditions of experimental site during spring and autumn seasons 2016

total carotenoid contents (TCC; $\mu\text{g/g}$), provitamin A contents (PVAC; $\mu\text{g/g}$) and non-provitamin A contents (NPVAC; $\mu\text{g/g}$). Total carotenoids contents (TCC) were estimated by spectrophotometer at 450nm absorption (Rodriguez-Amaya and Kimura, 2004). Spectrophotometric absorbance at 450 nm wavelength was used for estimation of TCC.

$$\text{Total Carotenoid Contents } (\mu\text{g/g}) = \frac{A_{\text{total}} \times \text{volume (ml)} \times 10^4}{A_{1\text{cm}}^{1\%} \times \text{Sample Weight (g)}}$$

Whereas, A_{total} = absorbance; volume = total volume of extract (25 mL); $A_{1\text{cm}}^{1\%}$ = absorption coefficient of 2500.

Provitamin A contents were quantified from randomly selected 20–30 seeds of self-pollinated lots by following the International Maize and Wheat Improvement Center (CIMMYT, in Spanish acronym) laboratory protocols for carotenoids analysis including extraction, separation and quantification by high-performance liquid chromatography (HPLC; Galicia *et al.*, 2009).

Statistical Analysis

Data were subjected to analysis of variance (Steel *et al.*, 1997). Data for line \times tester data were subjected to both Kempthorne's (1957) and GGE biplot analysis (Yan and Kang, 2003). Kempthorne's analysis (1957) was based on combining ability estimates of Sprague and Tatum (1942). Biometrical inferences (variance components, coefficients of variations, GCA and SCA variance, additive and non-additive variance) of Kempthorne's analysis were made by following the Singh and Chaudhary (1979). GCA and SCA effects from Kempthorne's analysis (1957) were graphically presented by using principal component analysis (PCA) biplots. Mid parent heterosis (MPH) and better parent heterosis (BPH) were also represented by PCA biplots.

$$\text{Mid parent heterosis (MPH)} = \frac{F_1 - \text{Mid parent}}{\text{Mid parent}} \times 100$$

$$\text{Better parent heterosis (BPH)} = \frac{F_1 - \text{Better parent}}{\text{Better parent}} \times 100$$

Two-way data tables were generated for all studied traits and subjected to combining ability estimates by using

GGE biplot analysis. Genotypic performance is mixed effect of genotype main effect (G), environmental main effect (E) and genotype \times environment interaction (GE) effects. However, two-way data tables like diallel cross data (Yan and Hunt, 2002), genotype-by-trait data and genotype-by-genetic marker data (Yan and Kang, 2003) could also be analyzed by GGE biplot analysis. Yan and Kang (2003) devised that tester-centered principal component analysis is the model used for biplot analysis of diallel data thus, similar data manipulations could also be accomplished for two-way data of line \times tester analysis. Average yield and genotype stability are equivalent to GCA and SCA effects of parents respectively for two-way line \times tester data. Model of GGE biplot analysis for line \times tester data is given as following:

$$Y_{ij} - b_j = a_1 e_{1i} h_{1j} + a_2 e_{2i} h_{2j} + e_{ij}$$

Where, Y_{ij} is genotypic values of the combination (pure inbred lines or F_1 hybrids) between inbred i and tester j for a given trait; b_j is average value of all combinations with tester j , a_1 and a_2 are singular values for PC_1 and PC_2 . e_{1i} and e_{2i} are eigenvector values of PC_1 and PC_2 for i th inbred (entry), h_{1j} and h_{2j} are eigenvectors values of PC_1 and PC_2 for j th tester, e_{ij} is the residual of model for i th inbred and j th tester.

Results

Significance of Differences

Analysis of variance (ANOVA) showed that genotypes, F_1 single cross hybrids (crosses), inbred lines, line \times tester interaction and contrast of parents versus crosses were significantly different for all studies traits. Parents and contrast of testers versus lines were insignificant for ASI. Testers were not significantly different for GPR (Table 1). Summary statistics showed that means of F_1 crosses were higher than parents for most of the studied agronomic, yield and carotenoid traits (Table 1).

Kempthorne's Analysis for Line \times Tester Data

Kempthorne's conventional analysis for line \times tester facilitated the estimation of GCA effects, SCA effects, variance component analysis, percent contribution of parents, percent contribution of crosses, mid parent heterosis and better parent heterosis for studies F_1 crosses.

Combining Ability Estimates

Numerical values for GCA and SCA effects based on the Kempthorne's analysis were presented by PCA biplots. Positioning of the genotypes with reference to the particular trait vector and central origin of the biplots are indicating the performance of genotypes *i.e.* farther away from origin towards positive side of vector is showing the highest mean value for combining ability effects.

Table 1: Mean squares and summary stat of different agronomic, yield contributing and carotenoid related traits for line × tester data of maize

SOV	DF	PH	EH	DS	DT	ASI	GPR	RPC	FW	YPH	TCC	PVAC	NPVAC
Replication	2	74	55	122.61	71.74	7.76	37.02	0.50	2.16	12.97	3.3	0.02	1
Genotypes	64	1457**	847**	28.61**	32.02**	1.76**	138.16**	8.02**	3.50**	54.20**	255.6**	6.86**	263**
Parents (P)	14	1392**	679**	42.52**	39.37**	0.39ns	173.64**	18.03**	7.39**	10.20**	149.4**	7.66**	99**
Crosses (C)	49	878**	723**	24.03**	29.39**	1.95**	36.07**	5.14**	2.46**	67.64**	238.8**	6.17**	244**
P vs C	1	30722**	9275**	58.55**	57.55**	11.82**	4643**	8.96**	0.39**	11.33**	2568.8**	29.61**	3467**
Lines (L)	9	910**	1129**	24.30**	35.78**	2.30**	30.67**	3.02**	1.33**	77.75**	126.1**	6.09**	123**
Testers (T)	4	3545**	817**	19.42**	21.15**	1.79**	11.76ns	1.94**	8.38**	16.61**	546.9**	22.87**	495**
L vs T	1	1007**	745**	9.34**	2.84*	0.09ns	309.88**	21.51**	90.18**	74.35**	1013.4**	79.94**	353**
L × T	36	574**	611**	24.47**	28.71**	1.88**	40.13**	6.03**	2.08**	70.79**	232.7**	4.34**	247**
Error	128	68	41	0.67	0.68	0.35	9.70	1.11	0.02	0.64	1.5	0.10	2
Summary Statistics for different agronomic and carotenoid traits													
Mean	Lines	161.03	82.03	61.37	58.13	2.48	24.83	13.60	3.669	10.43	19.40	4.09	14.41
	Testers	151.00	73.40	60.40	57.60	2.58	19.27	12.13	0.667	7.707	9.333	1.263	8.467
	F ₁ crosses	187.48	95.53	62.35	59.25	3.10	34.56	13.62	2.563	10.097	24.66	2.223	22.44
Minimum	Lines	123.33	73.00	52.33	47.67	2.00	10.67	9.333	2.530	8.000	12.33	3.000	7.000
	Testers	134.00	55.00	58.00	56.00	2.00	14.33	8.667	0.367	6.200	5.333	0.600	5.000
	F ₁ crosses	141.50	54.50	53.00	48.50	1.75	26.33	10.67	0.475	1.287	12.00	0.139	9.744
Maximum	Lines	192.33	104.33	66.33	62.67	2.83	37.33	18.67	4.933	12.00	27.00	5.667	22.33
	Testers	178.00	102.67	66.00	61.67	3.24	25.00	13.67	1.200	9.867	11.67	1.713	11.00
	F ₁ crosses	214.50	122.5	67.25	63.75	4.75	41.67	16.67	4.325	19.92	48.50	6.739	46.87

Where, PH: plant height (cm), EH: ear height (cm), DS: days to 50% silking, DT: days to 50% tesseling, ASI: anthesis silking interval, GPR: grains per row, RPC: rows per cob, FW: field weight (Kg), YPH: yield per hectare (tonnsha⁻¹), TCC: total carotenoid contents (µg/g), PVAC: provitamin A contents (µg/g), NPVAC: non-provitamin A contents (µg/g)

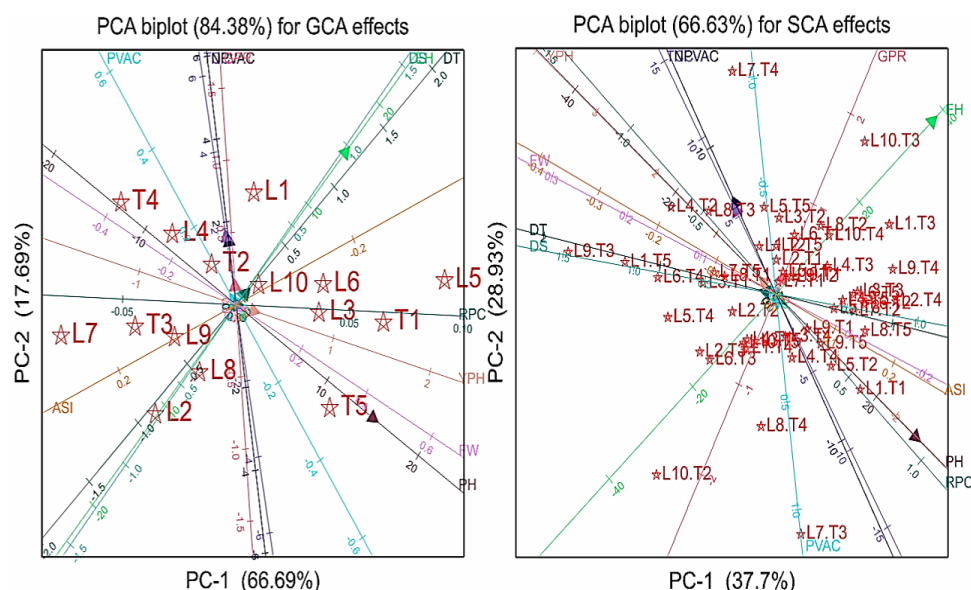


Fig. 2: PCA biplot for GCA and SCA effects of different agronomic, yield and carotenoid related traits. Where, PH: plant height (cm), EH: ear height (cm), DS: days to 50% silking, DT: days to 50% tesseling, ASI: anthesis silking interval, GPR: grains per row, RPC: rows per cob, FW: field weight (Kg), YPH: yield per hectare (tonnsha⁻¹), TCC: total carotenoid contents (µg/g), PVAC: provitamin A contents (µg/g), NPVAC: non-provitamin A contents (µg/g), L1: 15068, L2: 15076, L3: 15071, L4: 15163, L5: 24687, L6: 14959, L7: 14970, L8: 15205, L9: 24689, L10: 15350, T1: 15055, T2: 15052, T3: 15073, T4: 15130 and T5: 14966

Based on these biplots, briefly results for GCA effects of parents were described as following; L5, L3, T1 and T5 have highest GCA effects whereas, L7, L4, T4 and T3 have lowest GCA effects for PH and EH. L10, L6, L1 and T1 have highest GCA effects, whereas L9, L7, and T2 have lowest GCA effects for DS and DT. For ASI, L6, L1 and

T5 have lowest GCA effects. L1, L10, T2 for GRP; L5, L9 and T3 for RPC; L5, L8, L9, T1 and T5 for FW; L5, L8 and T1 for YPH had highest GCA effects. L1, L8, L9, T2 and T4 for TCC; L7, L3, L1, T1 and T3 for PVAC have highest GCA effects. L2, L4, L10, T1 and T5 have lowest GCA effects for NPVAC (Fig. 2).

Among studied F_1 hybrid combinations $L9 \times T3$, $L5 \times T5$ and $L4 \times T2$ have lowest SCA effects, whereas $L7 \times T3$, $L3 \times T3$ and $L1 \times T1$ have highest SCA effects for PH. For EH, $L10 \times T2$, $L7 \times T3$, $L9 \times T3$ and $L6 \times T3$ had lowest SCA effects, whereas $L10 \times T3$, $L1 \times T3$ and $L9 \times T4$ hybrids have highest SCA effects. Lowest SCA effects for DS were observed in crosses of $L5 \times T1$, $L7 \times T3$ and $L9 \times T4$ whereas, highest SCA effects were found in $L2 \times T3$, $L1 \times T4$, $L3 \times T5$ crosses. F_1 single cross of $L7 \times T3$, $L5 \times T1$ and $L9 \times T4$ had lowest SCA effects, whereas $L3 \times T5$, $L7 \times T1$, $L9 \times T2$ and $L2 \times T3$ had highest SCA effects for DT. For ASI, crosses of $L4 \times T3$, $L5 \times T4$, $L3 \times T5$ and $L1 \times T3$ have lowest SCA effects, whereas $L3 \times T3$, $L4 \times T4$, $L5 \times T1$ and $L10 \times T4$ crosses have highest SCA effects. Single cross hybrids with parental combinations of $L8 \times T1$, $L7 \times T2$, $L7 \times T5$ and $L10 \times T4$ for GPR; $L9 \times T1$, $L5 \times T2$, $L8 \times T4$ and $L4 \times T2$ for RPC; $L7 \times T2$, $L3 \times T5$, $L8 \times T4$ and $L4 \times T2$ for FW; $L8 \times T3$, $L5 \times T4$ and $L10 \times T5$ for YPH have highest SCA effects. Crosses between $L1 \times T1$, $L7 \times T1$, $L7 \times T3$ and $L1 \times T4$ have lowest SCA effects, whereas $L7 \times T4$, $L1 \times T5$, $L5 \times T4$ and $L8 \times T3$ have highest SCA effects for TCC. For PVAC, singles cross hybrids $L1 \times T1$, $L7 \times T3$, $L8 \times T4$, $L10 \times T4$ and $L2 \times T5$ have highest SCA effects. $L1 \times T1$, $L1 \times T4$ and $L8 \times T4$ hybrids have lowest, whereas $L8 \times T3$, $L5 \times T4$, $L7 \times T4$ and $L1 \times T5$ hybrids have highest SCA effects for NPVAC (Fig. 2).

Percent Contribution and Variance Analysis for Traits

Contribution of lines, testers and interaction of parents for particular traits was estimated to access the proportional effectiveness of different parents and cross combinations. Percent contribution for line \times tester interaction was 48.03% for PH, 62.08% for EH, 74.83% for DS, 71.77% for DT, 70.86% for ASI, 81.72% for GPR, 86.12% for RPC, 62.21% for FW, 76.88% for YPH, 71.61% for TCC, 51.63% for PVAC and 74.19% for NPVAC. Lines contributed more than testers with proportion of 28.70% for EH, 18.58% for DS, 22.36% for DT, 21.65% for ASI, 15.62% for GPR, 10.80% for RPC, 21.11% for YPH, 30.13% for PVAC and 16.28% for NPVAC (Table 2).

Phenotypic variances were 530.99 for PH, 309.88 for EH, 9.98 for DS, 11.13 for DT, 0.82 for ASI, 52.52 for GPR, 3.42 for RPC, 1.182 for FW, 18.49 for YPH, 86.19 for TCC, 2.35 for PVAC and 88.66 for NPVAC. Phenotypic variances were found to be higher than genotypic and environmental variances for all studied traits. Phenotypic and genotypic coefficient of variation (PCV and GCV) showed that PCV was also higher than GCV for all of the studied traits. PCV was 12.76 for PH, 19.19 for EH, 5.09 for DS, 5.66 for DT, 30.62 for ASI, 22.73 for GPR, 13.69 for RPC, 42.029 for FW, 43.15 for YPH, 40.95 for TCC, 62.98 for PVAC and 46.78 for NPVAC (Table 2). GCA variance, SCA variance, ratio of GCA to SCA variance, additive variance and dominance variance for all studies traits were also presented in Table 2. Dominance variances were

551.53 for PH, 596.87 for EH, 24.25 for DS, 28.48 for DT, 1.76 for ASI, 36.89 for GPR, 5.66 for RPC, 2.07 for FW, 70.57 for YPH, 232.25 for TCC, 4.32 for PVAC and 246.13 for NPVAC which were numerically higher than additive variances for all traits (Table 2).

Mid Parent Heterosis (MPH) and Better Parent Heterosis (BPH)

Heterosis estimates were measured numerically according to the formulae mentioned in materials and methods but to improve the comprehension these estimates were subjected to PCA biplot for graphical view. Distance of genotype from the origin of biplot towards positive axis of the trait vector reflected the positive heterosis whereas, towards negative side reflected the negative heterosis. Among studied single cross F_1 maize hybrids, $L6 \times T1$, $L6 \times T2$, $L6 \times T5$ and $L9 \times T5$ showed the highest positive whereas, $L8 \times T3$, $L9 \times T3$ and $L10 \times T3$ showed highest negative MPH for PH (Fig. 3). For ASI, $L10 \times T5$, $L3 \times T5$, $L6 \times T2$ and $L4 \times T3$ showed highest negative MPH. F_1 crosses of $L3 \times T3$, $L1 \times T1$, $L3 \times T1$ and $L10 \times T1$ presented the highest positive MPH for YPH. For TCC, crosses of $L2 \times T1$, $L5 \times T2$, $L8 \times T2$, $L1 \times T3$, $L3 \times T3$, $L4 \times T3$, $L5 \times T3$ and $L8 \times T3$ had highest positive MPH. Single cross hybrids of $L1 \times T1$, $L3 \times T1$, $L1 \times T3$, $L3 \times T3$, $L5 \times T3$ and $L7 \times T3$ had highest positive MPH for PVAC (Fig. 3). MPH estimates for all other traits could also be viewed from Fig. 3.

BPH estimates for all studies traits were graphically presented in Fig.3 however, results are described here only for selective traits. F_1 single crosses of $L6 \times T1$, $L9 \times T1$, $L6 \times T2$, $L6 \times T5$ and $L9 \times T5$ had highest positive BPH whereas, $L8 \times T3$, $L9 \times T3$, $L7 \times T4$ and $L2 \times T3$ had negative BPH for PH. $L4 \times T3$, $L6 \times T2$, $L6 \times T1$ had highest negative BPH for ASI. $L3 \times T1$, $L3 \times T3$, $L8 \times T3$, $L5 \times T4$ and $L10 \times T5$ had highest positive BPH for YPH. $L2 \times T1$, $L3 \times T2$, $L4 \times T2$, $L5 \times T2$, $L3 \times T3$ and $L5 \times T4$ had highest positive BPH for TCC. $L1 \times T1$, $L3 \times T1$, $L3 \times T3$, $L1 \times T3$, $L5 \times T3$ and $L7 \times T3$ have highest positive BPH for PVAC. $L1 \times T1$, $L7 \times T1$, $L7 \times T3$, $L1 \times T4$, $L6 \times T5$ and $L7 \times T5$ had negative BPH for NPVAC (Fig. 3).

GGE Biplot Analysis for Line \times Tester Data

GGE biplot analysis provides multiple characteristics features however, in present study with special perspective of line \times tester data most important features like, average tester coordinate (ATC) for accessing the GCA effects, average tester ordinate (ATO) for accessing the SCA effects and polygon view for accessing the best crosses were only manifested.

Average Tester and General Combining Ability (GCA) of Lines

Average tester coordinate (ATC) feature of GGE biplot enabled the assessment of GCA effects of testers.

Table 2: Contribution of lines, testers, crosses, variances and heritability estimates for agronomic, yield and carotenoid related traits

	PH	EH	DS	DT	ASI	GPR	RPC	FW	YPH	TCC	PVAC	NPVAC
Contribution of Line (L)	19.03	28.70	18.58	22.36	21.65	15.62	10.80	9.957	21.11	9.70	30.13	16.28
Contribution of Tester (T)	32.94	9.22	6.60	5.88	7.49	2.66	3.08	27.837	2.01	18.70	18.24	9.53
Contribution of L × T	48.03	62.08	74.83	71.77	70.86	81.72	86.12	62.206	76.88	71.61	51.63	74.19
Standard Error for Lines	2.13	1.66	0.21	0.21	0.15	0.80	0.27	0.038	0.21	0.31	0.08	0.32
Standard Error for Testers	1.51	1.17	0.15	0.15	0.11	0.57	0.19	0.027	0.15	0.22	0.06	0.23
Standard Error for L×T	4.76	3.71	0.47	0.48	0.34	1.80	0.61	0.085	0.46	0.70	0.18	0.72
Phenotypic Variance	530.99	309.88	9.98	11.13	0.82	52.52	3.42	1.182	18.49	86.19	2.35	88.66
Genotypic Variance	462.98	268.50	9.32	10.44	0.47	42.82	2.30	1.160	17.85	84.72	2.26	87.11
Environmental Variance	68.00	41.38	0.67	0.68	0.35	9.70	1.11	0.022	0.64	1.46	0.10	1.56
P coefficient V (PCV)	12.76	19.19	5.09	5.66	30.62	22.73	13.69	42.029	43.15	40.95	62.98	46.78
G coefficient V (GCV)	11.91	17.86	4.92	5.48	23.11	20.52	11.24	41.640	42.40	40.60	61.65	46.37
GCA variance (σ_{GCA}^2)	73.47	16.10	0.12	0.01	0.01	0.84	0.16	0.123	1.05	4.61	0.45	2.77
SCA variance (σ_{SCA}^2)	551.53	596.87	24.25	28.48	1.76	36.89	5.66	2.073	70.57	232.25	4.31	246.13
GCA var. /SCA var.	0.13	0.03	0.00	0.00	0.00	0.02	0.03	0.059	0.01	0.02	0.10	0.01
Additive V (σ_a^2)	146.93	32.21	0.23	0.02	0.01	1.68	0.32	0.247	2.10	9.22	0.90	5.55
Dominance V (σ_d^2)	551.53	596.87	24.25	28.48	1.76	36.89	5.66	2.073	70.57	232.25	4.31	246.13

Where, PH: plant height (cm), EH: ear height (cm), DS: days to 50% silking, DT: days to 50% tesseling, ASI: anthesis silking interval, GPR: grains per row, RPC: rows per cob, FW: field weight (Kg), YPH: yield per hectare (tonnsha⁻¹), TCC: total carotenoid contents (µg/g), PVAC: provitamin A contents (µg/g), NPVAC: non-provitamin A contents (µg/g)

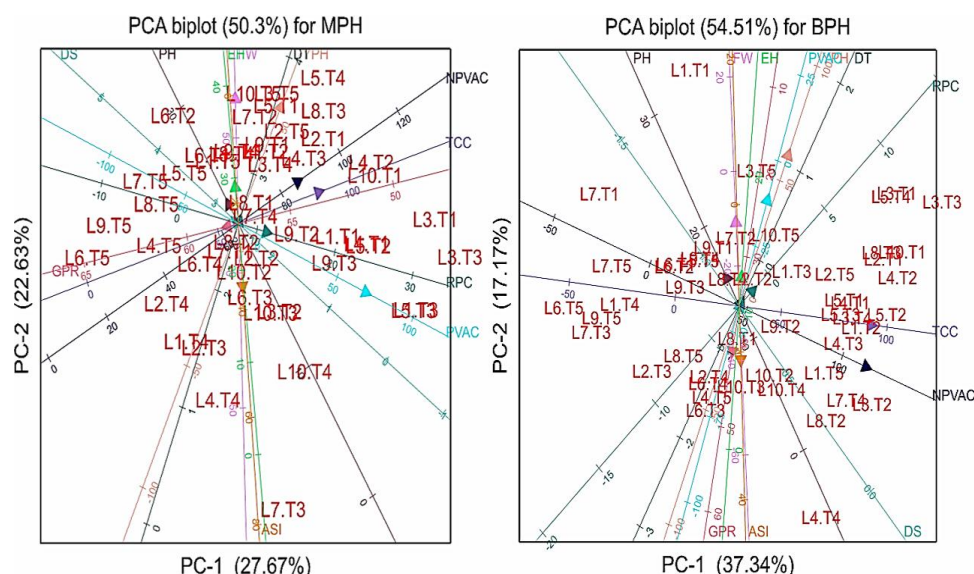


Fig. 3: PCA biplot for mid parent heterosis (MPH) and better parent heterosis (BPH) of different agronomic, yield and carotenoid related traits. Where, PH: plant height (cm), EH: ear height (cm), DS: days to 50% silking, DT: days to 50% tesseling, ASI: anthesis silking interval, GPR: grains per row, RPC: rows per cob, FW: field weight (Kg), YPH: yield per hectare (tonnsha⁻¹), TCC: total carotenoid contents (µg/g), PVAC: provitamin A contents (µg/g), NPVAC: non-provitamin A contents (µg/g), L1: 15068, L2: 15076, L3: 15071, L4: 15163, L5: 24687, L6: 14959, L7: 14970, L8: 15205, L9: 24689, L10: 15350, T1: 15055, T2: 15052, T3: 15073, T4: 15130 and T5: 14966

Small circle on ATC is the position of average tester which is defined by average of principal components (PC1 and PC2) for all testers. The line passing through the average tester and origin of biplot, with an arrow pointing to average tester, is called the average tester axis (ATA) or ATC abscissa however, line passing through the biplot origin and perpendicular to the average tester axis (ATA) is called average tester ordinate (ATO) or ATC ordinate. GCA and SCA effects are described as a features of parental lines.

GCA effects are determined by projection of parental lines on the ATC abscissa. Ranking of parental lines based on GCA effects is defined by the lines parallel to ATC ordinate.

GGE ranking biplots for all traits were developed separately. Ranking biplot was reflecting the 72.23% (PC1: 42.10%, PC2: 30.12%) variability for PH. Average tester axis showed that T4 and T5 were average testers, T1 was below average and T2 was above average tester for PH.

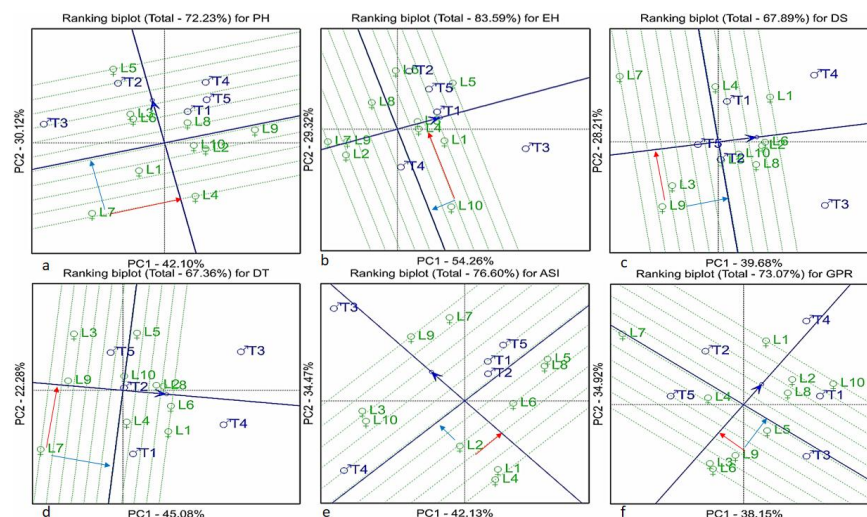


Fig. 4: Average Tester Coordination (ATC) view of GGE biplot for line × tester data. ATC abscissa represented by blue arrow pointing the average tester whereas, red arrow projections on the ATC ordinate representing the SCA effects of entries. (a) Plant height (PH; cm), (b) Ear height (EH; cm), (c) Days to 50% silking (DS), (d) Days to 50% tassel (DT), (e) Anthesis silking interval (ASI), (f) grains per row (GPR), L1: 15068, L2: 15076, L3: 15071, L4: 15163, L5: 24687, L6: 14959, L7: 14970, L8: 15205, L9: 24689, L10: 15350, T1: 15055, T2: 15052, T3: 15073, T4: 15130 and T5: 14966

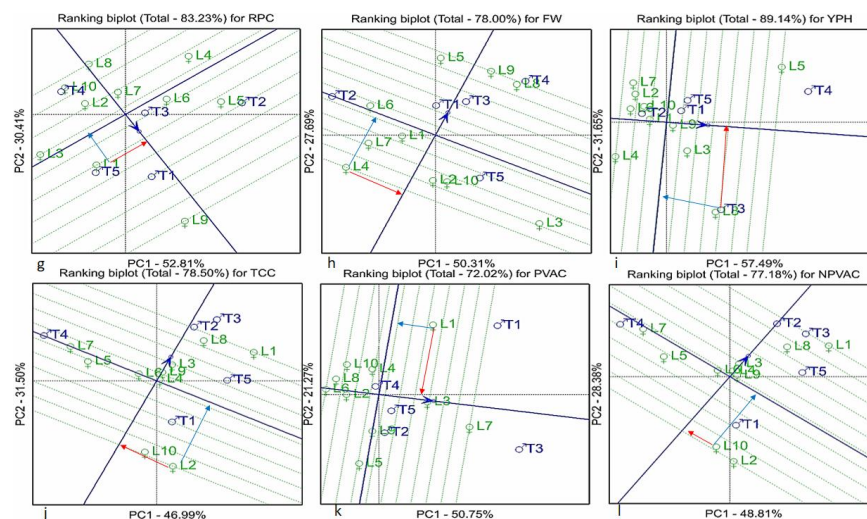


Fig. 4: Average Tester Coordination (ATC) view of GGE biplot for line × tester data. ATC abscissa represented by blue arrow pointing the average tester whereas, red arrow projections on the ATC ordinate representing the SCA effects of entries. (g) Rows per cob (RPC), (h) Field weight (FW; Kg), (i) Yield per hectare (YPH; tonss), (j) Total carotenoid contents (TCC; µg/g), (k) Provitamin A contents (PVAC; µg/g) and (l) Non-provitamin A contents (NPVAC; µg/g), L1: 15068, L2: 15076, L3: 15071, L4: 15163, L5: 24687, L6: 14959, L7: 14970, L8: 15205, L9: 24689, L10: 15350, T1: 15055, T2: 15052, T3: 15073, T4: 15130 and T5: 14966

For PH, L5, L7 and L4 were having higher GCA effects and L9, L8 and L10 were having lower GCA effects (Fig. 4). GGE ranking biplot reflected the 67.89% (PC1: 39.68%, PC2: 28.21%) for DS, 67.36% (PC1: 45.08%, PC2: 22.28%) for DT and 76.60% (PC1: 42.13%, PC2: 34.47%) variability for ASI. T1 was average tester for DS and DT. T1 and T5 were average testers for ASI. T3 and T4 were below average testers and T5 was above average tester for

DS and DT. T3 was above average tester and T2 and T4 were below average testers for ASI (Fig. 4).

GGE ranking biplot depicted the 78.00% (PC1: 50.31%, PC2: 27.69%) and 89.14% (PC1: 57.49%, PC2: 31.65%) variability for FW and YPH respectively. Average testers were T1 and T3 for FW; T1 and T5 for YPH. T4 was above average tester for FW; T3 and T4 were above average testers for YPH (Fig. 4).

GGE ranking biplot showed the 78.50% (PC1: 46.99%, PC2: 31.50%), 72.02% (PC1: 50.75%, PC2: 21.27%) and 77.18% (PC1: 48.81%, PC2: 28.38%) variability for TCC, PVAC and NPVAC respectively. T5 was average tester for TCC and PVAC whereas, T4 was average tester for NPVAC. T2 and T3 were above average testers for TCC and NPVAC. T1 and T3 were above average testers for PVAC (Fig. 4).

Specific Combining Ability (SCA) of Lines

SCA effects are associated with crosses however, could not be explained similar to the GCA effects. Biplot facilitates the visualization of SCA effects of parents. ATC abscissa dictates the GCA effects then ATC ordinate being orthogonal to the GCA effects approximate the SCA effect. SCA effects in GGE biplot showed the tendency of hybrids to produce superior hybrids. ATC ordinate in GGE average tester ranking biplot, was used as reflector of SCA effects for entries. Farther away the projection from the origin, higher is the SCA effects for entries whereas, projection closer to origin are depicting least SCA effects for entries.

L9, L7 and L2 were having higher SCA effects whereas, L3, L5 and L6 were having lower SCA effects for PH (Fig. 4). L4, L9, and L7 for DS; L7, L5, L3 and L4 for DT and L5, L8, L7 and L10 were having higher SCA effects for ASI (Fig. 4). L7, L10 and L5 for GPR; L4, L3 and L5 for RPC were having highest SCA effects. For YPH, L9 and L1 were having lowest SCA effects whereas, L8 and L5 were having highest SCA effects. L7, L5, L1 and L2 for TCC; L1, L5 and L9 for PVAC; L2, L1, L10 and L7 for NPVAC were having highest SCA effects (Fig. 4).

Best Cross

Polygon view of GGE biplot enabled the visualization of best crosses between the tester and entries. Polygon consisted of the straight line connecting the entries (inbred lines) located farthest from the biplot origin so that all other entries are located within polygon. Biplot is divided into sectors by drawing the lines perpendicular to each side of the polygon. Sectors are named according to the names of entries present at the vertex of polygon view. For PH, polygon view divided the biplot into four different sector named as L9, L5, L7 and L4 sectors. In L9 sector, three testers i.e. T1, T4 and T5 were present, which showed that L9×T1, L9×T4 and L9×T5 were best crosses. In L5 sector, two testers i.e. T2 and T3 were present, which depicted that L5×T2 and L5×T3 were best cross for PH. On the other hand, in L7 and L4 sector, there was no tester thus entries present in these sectors produced poor hybrids with some or all of these five testers (Fig. 5).

Polygon view for DS, categorized the entries into five sectors named L1, L4, L7, L9 and L8. Based on sectoral distribution of entries and tester, L1×T4, L1×T1, L4×T1, L9×T2, L9×T5, L8×T2, L8×T3 were best crosses.

For DT, L5×T3, L3×T5, L7×T1, L1×T1, L1×T4, L6×T3 were best crosses. L5×T5, L5×T1, L5×T2, L9×T3, L10×T4 were best crosses for ASI. L5×T4, L5×T5, L5×T1, L7×T2, L8×T3 were best crosses for YPH. L1×T2, L1×T3, L1×T5, L8×T2, L8×T3, L8×T5, L7×T4, L1×T1, L2×T1 were best crosses for TCC. L1×T1, L1×T4, L5×T2, L7×T3, L7×T5 were best crosses for PVAC. L1×T3, L1×T2, L1×T5, L8×T3, L8×T2, L8×T5, L7×T4, L10×T1, L2×T1 were best crosses for NPVAC (Fig. 5). Similarly best crosses for all other traits could also be identified from polygon view of biplots (Fig. 5).

Discussion

High level of variability for agronomic, yield, carotenoids and provitamin A traits was observed in single cross yellow maize hybrids. This variability was the ultimate result of current hybridization of parents using line into tester mating fashion (Liu *et al.*, 2003). Variability in different traits was also evidently reported by Egesel *et al.* (2003); Menkir and Maziya-Dixon (2004); Chander *et al.* (2008); Menkir *et al.* (2008); Pixley *et al.* (2011); Wurtzel *et al.* (2012); Owens *et al.* (2014). Observed variability was indicating that there is potential for genetic improvement of maize germplasm for subjected traits. Variation in means were also previously observed for different agronomic traits, yield components, and nutritional quality traits (Balci *et al.*, 2004; de Almeida Rios *et al.*, 2014; Werle *et al.*, 2014).

Single cross yellow maize hybrids in present study showed that almost 90% of the total carotenoid contents were non-provitamin A contents. Results of Hwang *et al.* (2016) showed that 72% of the total carotenoids were non-provitamin A carotenoids. Alfieri *et al.* (2014) reported that non-provitamin A carotenoids were proportionally higher even in biofortified maize hybrids. Average of 50 single crossed maize hybrids has 3.22 µg/g provitamin A contents which were not equivalent to first generation biofortified maize hybrids. Pfeiffer and McClafferty (2007) reported that maize hybrids having less than 4.0 µg/g provitamin A contents were described as non-biofortified whereas, hybrids with range of >4–8 µg/g provitamin A contents were classified as first generation biofortified maize hybrids. However, according to the recommendations of HarvestPlus, 15 µg/g provitamin A contents was final target to be attained for biofortification of maize. These differences in the provitamin A contents may be attributed to the differences in the selection for total provitamin A during development of inbred lines, general differences between temperate and tropical environments, and genotype × environment interaction.

Kempthorne's analysis was conventionally used for combining ability analysis of line × tester data which facilitated the assessment of combining ability, heterosis and genetic variances. Desirability of GCA effects determined the selection of positive or negative GCA effects.

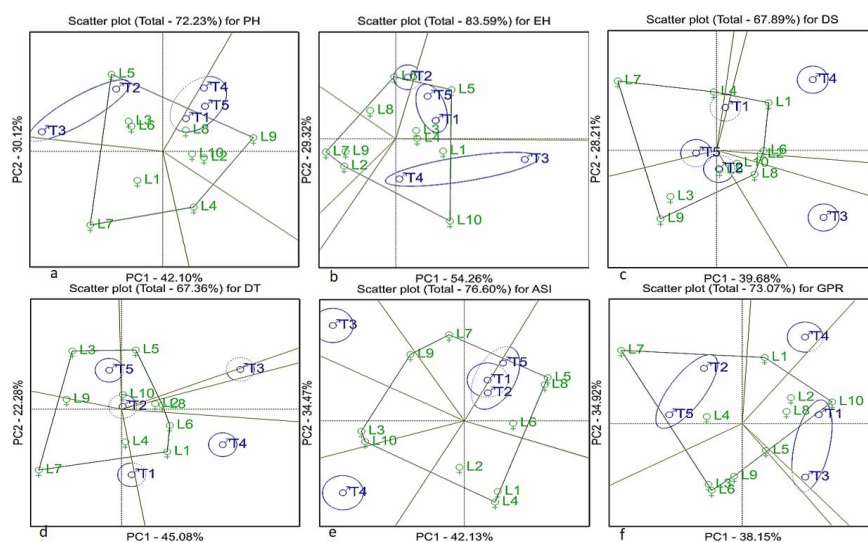


Fig. 5: Scatter plot view of GGE biplot for line \times tester data. Blue spheres enclosing the testers representing the heterotic groups. Polygon view representing the best crosses. (a) Plant height (PH; cm), (b) Ear height (EH; cm), (c) Days to 50% silking (DS), (d) Days to 50% tasseling (DT), (e) Anthesis silking interval (ASI), (f) grains per row (GPR), L1: 15068, L2: 15076, L3: 15071, L4: 15163, L5: 24687, L6: 14959, L7: 14970, L8: 15205, L9: 24689, L10: 15350, T1: 15055, T2: 15052, T3: 15073, T4: 15130 and T5: 14966

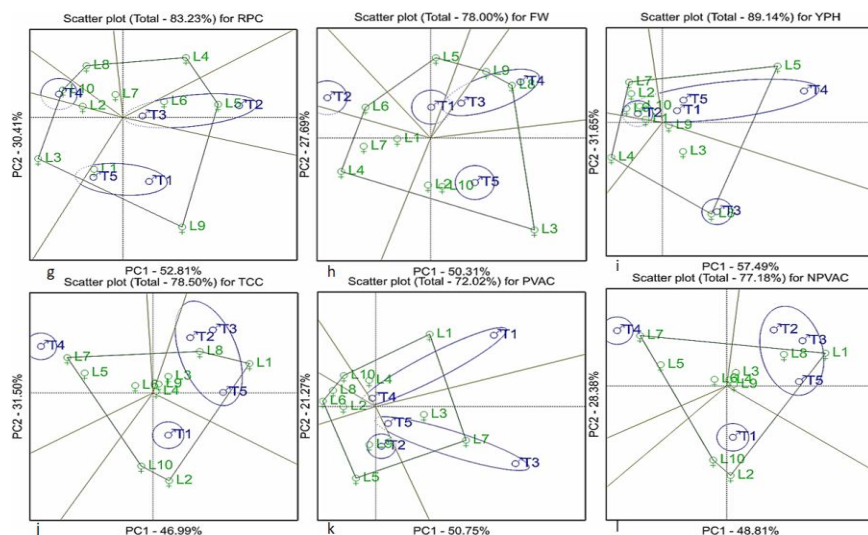


Fig. 5: Scatter plot view of GGE biplot for line \times tester data. Blue spheres enclosing the testers representing the heterotic groups. Polygon view representing the best crosses. (g) Rows per cob (RPC), (h) Field weight (FW; Kg), (i) Yield per hectare (YPH; tonss), (j) Total carotenoid contents (TCC; $\mu\text{g/g}$), (k) Provitamin A contents (PVAC; $\mu\text{g/g}$) and (l) Non-provitamin A contents (NPVAC; $\mu\text{g/g}$), L1: 15068, L2: 15076, L3: 15071, L4: 15163, L5: 24687, L6: 14959, L7: 14970, L8: 15205, L9: 24689, L10: 15350, T1: 15055, T2: 15052, T3: 15073, T4: 15130 and T5: 14966

GCA effects for different agronomic and yield components were studied previously by large number of researchers and they reported both positive and negative GCA effects for different traits as observed in present study (Egesel *et al.*, 2003; Menkir and Maziya-Dixon, 2004; Chander *et al.*, 2008; Menkir *et al.*, 2008; Pixley *et al.*, 2011; Wurtzel *et al.*, 2012; Owens *et al.*, 2014;

Kahrman *et al.*, 2016). SCA effects were also widely different for different crosses and traits as both positive and negative effects were prevailing. Variability in the SCA effects showed that there were large number of opportunities in terms of specific cross combinations for desirable genetic improvement of the studied traits (Egesel *et al.*, 2003; Balci *et al.*, 2004;

Menkir and Maziya-Dixon, 2004; Chander *et al.*, 2008; Menkir *et al.*, 2008; Pixley *et al.*, 2011; Wurtzel *et al.*, 2012; Owens *et al.*, 2014; Werle *et al.*, 2014; de Almeida Rios *et al.*, 2014; Kahrman *et al.*, 2016).

Combining ability estimation for provitamin A contents was very important for understanding the genetic basis of this trait. SCA effects and non-additive variance were most prevalent for provitamin A contents in studied single cross yellow maize hybrids. Egesel *et al.* (2003) reported significant GCA and SCA effects but observed the preponderance of GCA effects for β -carotene. Suwarno *et al.* (2014) reported the predominance of SCA effects for total provitamin A contents and β -cryptoxanthin.

L5 (24687), L6 (14959) and T1 (15073) were having high GCA effects for YPH, RPC and FW. L1, L5 and T4 were having high GCA effects for TCC, PVAC and NPVAC. L7×T3 (14970 × 15073), L8×T4 (15205 × 15130), L5×T2 (24687 × 15052) were having high SCA effects for PVAC. L2×T5 (15076 × 14966), L3×T3 (15071 × 15073), L4×T2 (15163 × 15052) were having high SCA effects for YPH. L3×T3 (15071 × 15073) had high SCA effects for YPH, TCC, PVAC and NPVAC. L8×T4 (15205 × 15130) had high SCA effects for PVAC, RPC and FW. L2×T5 (15076 × 14966) had high SCA effects for PVAC, YPH, NPVAC and TCC. Halilu *et al.* (2016) also reported the significant differences in GCA and SCA effects of agronomic and carotenoid traits of maize hybrids by following the diallel mating design.

Contributions of lines, testers and their interaction showed that most of the variability was contributed by interaction of lines and testers. These findings showed that specific parental combinations could effectively be exploited for genetic improvement of studied traits in present research. Percent contribution also showed that studied lines were more contributors of variability than testers as it was also reported by Kahrman *et al.* (2016). PV and PCV were higher than corresponding GV and GCA respectively for all studied traits which showed the prevalence of environmental factors. Phenotypic and genotypic coefficient of variations were similar for DS, DT, TCC and NPVAC. Equivalence of phenotypic and genotypic coefficients of variations showed the predominance of repulsion phase of linkage for these traits. Resembling patterns of PCV and GCV were also reported by Prema *et al.* (2011); Ghosh *et al.* (2010); Ahsan *et al.* (2013).

Dominance variance was higher than additive variance all of the studied traits which strongly suggested the manipulation of heterosis breeding for genetic improvement of studied traits. These findings were agreed with previously reported results of Saleem *et al.* (2002), Amit and Joshi (2007). Kahrman *et al.* (2016) found that additive gene action was controlling the total carotenoid contents whereas, current study showed the prevalence of non-additive gene action. Non-additive gene action was also previously reported for plant height, number of kernels per row, grain

yield per plant (Zia and Chaudhary, 1980; Saghir, 1984), number of days taken to silking (Saghir, 1984; Naveed, 1989), number of days taken to tesseling (Naveed, 1989). Additive gene action was previously reported for number of kernel rows per ear and number of kernels per row (Saghir, 1984; Bukhari, 1986), grain yield, plant height (Bukhari, 1986). Halilu *et al.* (2016) found the predominance of additive gene action for β -cryptoxanthin and non-additive gene action for grain yield, β -carotene, α -carotene, and provitamin A. Egesel *et al.* (2003); Chander *et al.* (2008); Suwarno *et al.* (2014) found that both additive and non-additive gene actions were controlling the inheritance pattern of carotenoids in endosperm of maize. Preponderance of non-additive gene action in present study is strongly recommending the manipulation of heterosis breeding in maize.

Positive, negative and zero mid parent and better parent heterosis were observed in studied cross combinations of different agronomic, yield and carotenoid traits which was also previously reported by different researchers (Bekele and Rao, 2013; Kahrman *et al.*, 2016). L3×T3 (15071×15073), L5×T4 (24687×15130), L3×T1 (15071×15055), L4×T3 (15163×15073), L2×T1 (15076×15055), L8×T3 (15205×15073), L5×T2 (24687×15052) for RPC and TCC were having highest BPH. L1×T1 (15068×15055), L3×T5 (15071×14966), L7×T1 (14970×15055), L10×T5 (15350×14966), L7×T2 (14970×15052), L9×T1 (24689×15055) for FW, YPH and PVAC were having highest BPH. Ali *et al.* (2014); Dorina and Viorica (2015) and Amanullah *et al.* (2011) also studied the heterosis estimates for difference traits of maize and also found the gigantic variability in the heterosis estimates for different cross combinations.

In present study, we manipulated the GGE biplot analysis for line × tester data to visualize the combining ability effects of parents and crosses (Yan and Kang, 2003). Different testers performed as average tester for different agronomic, yield and carotenoid traits as visualized by GGE biplot analysis. It was evident that T5 (14966) was average tester for PVAC and YPH among studied five different testers. Ruswandi *et al.* (2015) also visualized the average testers for different agronomic traits of maize using GGE biplot analysis for line × tester data and found that different testers were average testers for different traits.

SCA effects are commonly associated with crosses however, GGE biplot facilitated the visualization of SCA effects for parents. ATC abscissa dictated the GCA effects whereas, ATC ordinate approximated the SCA effects (Yan and Kang, 2003). It was evident in present study that different lines were having different SCA effects for different studied traits. However, L1 (15068), L5 (24687) and L9 (24689) were having highest SCA effects for PVAC and YPH. Differences in the SCA effects are attributed to the differences in the heterotic potential of different cross combinations and differences in the genetic background of the genotypes. Ruswandi *et al.* (2015) also observed the

SCA effects of lines for different agronomic traits of maize using GGE biplot analysis for line \times tester data and found that different lines were having different SCA effects for different traits.

Polygon view of GGE biplot enabled the visualization of best cross combinations (Yan and Kang, 2003). It was found in present study that different testers were located in different sectors for different traits thus different cross combinations were best for different traits. L1 \times T1 (15068 \times 15055) and L1 \times T4 (15068 \times 15130) for PVAC whereas, L5 \times T1 (24687 \times 15055), L5 \times T5 (24687 \times 14966) and L5 \times T4 (24687 \times 15130) were best crossed for YPH.

Conclusion

Hybridization by using line \times tester mating design harbored the significant genetic variability in the crosses for different traits showing that availability of gigantic potential for genetic improvement. GCA and SCA estimates were different for different traits as shown by both Kempthorne's and GGE biplot analysis. L5, L6 and T1 had high GCA effects for YPH, RPC, and FW whereas, L1, L5 and T4 had high GCA effects for TCC, PVAC and NPVAC. Among studied crosses, L7 \times T3, L8 \times T4 and L5 \times T2 had high SCA effects for PVAC whereas, L2 \times T5, L3 \times T3 and L4 \times T2 had high SCA effects for YPH. L3 \times T3 has high SCA effects for YPH, TCC, PVAC and NPVAC. L8 \times T4 had SCA effects for PVAC, RPC and FW. L2 \times T5 had high SCA effects for PVAC, YPH, NPVAC and TCC. Results of Kempthorne's analysis and GGE biplot analysis were comparable for estimation of GCA and SCA effects. GGE biplot could be preferred for providing visual explanation of the effects.

Most of variability was contributed by line \times tester interaction which showed performance of parents could be evaluated only in specific cross combinations. Gene action showed that all of the traits were under the control of non-additive gene action, which strongly directs the manipulation of heterosis breeding for genetic improvement of traits. L3 \times T3, L5 \times T4, L3 \times T1, L4 \times T3, L2 \times T1, L8 \times T3, L5 \times T2 for RPC and TCC, whereas L1 \times T1, L3 \times T5, L7 \times T1, L10 \times T5, L7 \times T2, L9 \times T1 for FW, YPH and PVAC were having highest better parent heterosis.

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