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Combining Ability and Hybrid Potential of Selected Elite Advanced Breeding Lines with Parents (as Testers) of Commercial Hybrids in Bitter Gourd (*Momordica charantia* L.)

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Abstract

Evaluation of experimental inbreds for general combining ability (GCA) enables plant breeders to discard undesirable experimental advanced breeding lines (ABLs) and identify those that are potential for the development of superior hybrids. For evaluating ABLs for test cross (TC) hybrid potential, choice of tester (s) is critical. In this context, 10 ABLs selected based on their acceptable marketable fruit size and length were evaluated for their GCA for fruit yield plant^{-1}, fruit diameter and fruit length considering the performance of the hybrids derived from crossing ABLs with parents of elite hybrids as testers. The analysis of variance revealed significant differences among the ABLs for their *per se* performance as well as their general combining ability (GCA). Though testers themselves did not differ for their GCA, they significantly discriminated the ABLs with respect to their GCA and variance of TC hybrids. The most desirable testers were identified for the three traits based on their ability to discriminate the ABLs, toproduce TC hybrids with high trait mean and high GCA. The non-significant correlation between *per se* performance and GCA effects suggested that *per se* performance of ABLs is not a good indicator of their GCA effects for any of the three traits. However, significant positive and fairly high magnitude of correlation between *per se* performance of TC hybrids and sum of parental GCA effects for all the three traits indicated that parental GCA effect is a good indicator of *per se* performance and heterosis of TC hybrids. Prediction of hybrid heterosis based on parental GCA effects would save substantial resources as it enables the evaluation of only a few hybrids that are predicted to be most promising ones.

Keywords : Advanced breeding lines, Correlation, General combining ability, Test cross

Presence of monoecious inflorescence which offer ease of pollination control coupled with large number of seeds per emasculation and pollination as well aslow seed rate makes single cross hybrids (SCHs), a commercially viable cultivar option in bitter gourd. Development of SCHs offer good opportunity to combine earliness coupled with high fruit yield plant^{-1}, acceptable fruit quality and resilience to biotic stress(es) specific to producing environments to which hybrids are targeted. Heterotic SCHs are typically crosses between inbred lines developed from complementary heterotic groups (HGs). A large number of new inbred lines from complementary HGs are routinely developed in hybrid crop breeding programmes. Inbred lines of one HG need to be evaluated in hybrid combinations with inbred lines (as testers) of complementary HGs. The number of such hybrid combinations is often would be very large whose evaluation is time consuming and logistically inefficient given the limited resources. This challenge has long been recognised and hence different methods have been suggested and used for identifying and eliminating poorer inbred lines and focus on intense evaluation of limited number of promising inbred lines. The most attractive and widely used method for this purpose is to assess new experimental inbred lines for their general combining ability (GCA), which enable breeders to discard inbred lines with poor potential in hybrid combinations without much risk of losing potential inbred lines. Further, it is possible that the new experimental inbred lines might perform better with one tester and worse with a different tester. In other words, when multiple testers are used, there is a need to account for differences among the test inbred lines. Under these circumstances, GCA offers the most convenient and an objective criterion to select test inbred lines with good potential in hybrid combinations (Bernardo, 2020 and Bernardo, 2023). Only those inbred lines with good GCA need to be evaluated in hybrid combinations with testers and identify the best hybrids for commercial exploitation. This is because, *per se* performance of parents need not always be a good indicator of their GCA effects (Ali *et al*., 2011; Ai Zhi & Zheng, 2012; Hosana *et al*., 2015 and Fasahat *et al*., 2016).

Besides offering an unambiguous criterion for selecting parents, the parental GCA would serve as a powerful predictor of hybrid performance in the absence of significant hybrid specific combining ability (SCA) effects. In addition to high gca in desirable direction, the inbreds should also exhibit high test cross (TC) hybrid potential for identifying heterotic hybrids. For evaluating ABLs for TC hybrid potential, choice of desirable tester (s) is critical. In this backdrop, the objectives of the present study were to (i) identify desirable testers and (ii) assess GCA and TC hybrid potential of selected elite advanced breeding lines (ABLs) with parents of elite commercial hybrids as testers.

Material and Methods

Basic Genetic Material

Ten advanced breeding lines (ABLs) chosen from F_6 population derived from a bi-parental cross constituted the basic genetic material for the present study (Table 1). These ten ABLs were chosen based on their marketable fruit size and length. The parents of elite commercial hybrids namely, 83-016, 83-023, 83-028, 83-030 & 83-031 were usedas testers to assess gca and TC hybrid potential of ABLs (Table 1).

Table 1

Pedigree of advanced breeding lines and testers used in the study

Advanced breeding lines	Pedigree	Source
Female lines		
ABTG-03	8009/3-P1	Jhalari
ABTG-08	8117/8-P2	US-555
ABTG-09	8117/2-P2	Hirakani
ABTG-10	8117/2-P1	Hirakani
83-009	AVBG-1304/13B	AVRDC
83-010	AVBG-1310	AVRDC
83-012	AVBG-1327	AVRDC
83-018	BG-08F	Jyothi
83-020	BTG-57/4F	US-444
83-025	AVBG-1323B	US-555
Male lines (Testers)		
83-016	1315M	Jyothi
83-023	PALEE MALE	Palee
83-028	BTG-63M	CO-1
83-030	WL-33 FEMALE	White Long
83-031	BT-23B	Hirkani

Development of Experimental Material

The seeds of 10 ABLs and five testers were planted in the nursery. The 25 days-old healthy seedlings were transplanted to a crossing block. The 10 ABLs were crossed to 5 testers in a line × tester mating design to produce 50 test cross (TC) hybrids. The resultant 50 TC hybrids, their parents and one commercial check, Mayura constituted the experimental genetic material.

Field Evaluation of Experimental Genetic Material

The seeds of 50 TC hybrids and those of parents and check were sown in nursery. The standard package of practices was followed to raise healthy seedlings in the nursery. Five healthy 25 days-old seedlings of 50 TC hybrids and check Mayura were transplanted in two- replicated alpha lattice design at experimental plots of research and developmental station, ORBI Seeds International Private Limited, Sadahalli, Bengaluru during 2023 rainy season. Five healthy 25 days-old seedlings of parents were also transplanted in two replicated randomized block design in a separate trial during 2023 rainy season. The seedlings of each TC hybrid, check and parents were transplanted in a single row of three metres in length, with a spacing of one metre between plants and two metres between rows. To establish a healthy crop, the recommended management practices were followed during the crop growth phase.

Sampling of Plants and Data Collection

The data was recorded on all the five plants of each of the 50 TC hybrids and the check, Mayura and parents in each replication for fruit diameter, fruit length and fruit yield plant^{-1}. The marketable fruits were harvested in eight pickings from all the five plants from each TC hybrid and check. These fruits were weighed and expressed as fruit yield plant^{-1} (kg). In each TC hybrid, three fruits were selected randomly and were cut transversely and the diameter was measured in the middle portion of the cut-fruits using a standard scale. The data was averaged across three fruits and expressed as average fruit diameter (cm). For the same three fruits, the length was measured using a standard scale and expressed as average fruit length (cm). The data was also recorded on parents for these three traits.

Statistical Analysis

The replicated mean data of TC hybrids, parents and check was used for statistical analysis. Data of TC hybrids was subjected to combining ability analysis following line × tester mating design linear model (Kempthorne, 1957). The analysis was implemented using 'INDOSTAT' software version 9.1. The gca effects of 10 ABLs and five testers were estimated and their statistical significance was examined using 't' test.

Efficiency of the testers to discriminate the ABLs was quantified as variance of TC hybrids. As a first step towards estimating TC hybrids' variance, separate ANOVA was performed for two replicated data of 10 TC hybrids derived from each of the five testers based on the linear model of alpha lattice design (Patterson and Williams, 1976). The total variability among 10 TC hybrids derived each of the five testers was partitioned into sources attributable to hybrids, replications and residual. Analysis was implemented using 'R' studio version 4.3.3. Variance of 10 TC hybrids produced by each of the five testers was estimated by subtracting residual means squares from TC hybrid mean squares and dividing by number of replications. The homogeneity of variances of TC hybrids derived from each of the five testers was examined using Bartlett chi-square statistic (Bartlett, 1937). Significant and larger TC hybrid variance was considered as evidence for greater discriminating ability of the testers. The mean of TC hybrids was also estimated. The testers which produced TC hybrids with greater mean, GCA effects and variance were considered as most desirable ones.

Relationship of GCA Effects of Inbred Lines with their *per se* Performance

Relationship between *per se* performance of 10 ABLs and their GCA effects were determined by estimating Pearson's Rank Correlation Coefficient for three quantitative traits (QTs). High magnitude of positive significant and non-significant correlation indicates good and poor predictability of GCA effects of ABLs based on their *per se* performance.

Relationship of Hybrid *per se* Performance with Sum of Parental GCA Effects

Pearson's correlation coefficients between hybrids *per se* performance and sum of GCA effects of their parents were estimated for three QTs (Schrag *et al.*, 2009). Significant correlation with fairly high magnitude was interpreted as high predictability of hybrid *per se* performance based on sum of their parental GCA effects.

Estimation of Standard Heterosis

The standard heterosis for all TC hybrids was estimated using the commercial check, Mayura. On the basis of standard heterosis, the best two TC hybrids were chosen.

RESULTS AND DISCUSSION

Analysis of Variance (ANOVA)

ANOVA indicated significant differences among the TC hybrids for all the three QTs (Table 2). Significant mean squares attributable to lines and interaction of lines with testers suggested substantial variability for GCA effects of lines and sca effects of their crosses for all the three QTs. The mean squares attributable to lines were of a larger magnitude than those of testers and line × tester interaction for all the three QTs indicating greater contribution of the lines than the testers towards total variation among the TC hybrids. Several researchers such as Sowmya and Gangappa (2018), Biradar *et al*. (2020) and Kavya *et al*. (2023) have also reported greater contribution of lines than those of testers and line × tester interaction in maize. Non-significant mean squares due to testers indicated their comparable GCA effects for all the three traits. These results could be due to sharing of similar alleles at loci controlling the three QTs among the testers. However, a few testers with significant GCA effects for fruit yield plant^{-1} was evident (Table 3). Such results are not surprising given that 'F' test is less sensitive to detect significant differences among all the five testers and that significance of at least one tester for the target statistic such as GCA effects cannot be ruled out.

Identification of Desirable Testers

Desirable testers are the ones which efficiently discriminate the ABLs, produce high TC hybrid mean and high gca in desirable direction for the target traits.

Desirable Testers Based on Discriminating Ability

The tester, 83-016 followed by 83-031 and 83-030 with significantly greater variance (Table 3) discriminated the ABLs better than the other two testers for fruit yield plant^{-1}. For other two traits, namely fruit diameter and fruit length, the tester 83-016 showing significantly greater variance discriminated the ABLs better than other three testers. Thus, based on the criterion of TC hybrids' variance, the tester 83-016 could be considered as the most

TABLE 2

Analysis of variance of TC hybrids derived from five testers for fruit yield plant^{-1} and its component traits

Source of variation	Degrees of freedom	Mean sum of squares		
		Fruit yield plant^{-1} (kg)	Fruit diameter (cm)	Fruit length (cm)
Replications	01	0.006	10.24 ***	0.01
Hybrids	49	0.54 ***	16.51 ***	0.26 ***
Line effects	09	1.27 **	70.66 ***	0.65 **
Tester effects	04	0.45	1.73	0.15
Line *Tester effects	36	0.37 ***	4.61 ***	0.18 *
Error	49	0.001	0.64	0.64
Contribution of Line (%)	-	43.08	45.18	78.63
Contribution of Tester (%)	-	6.78	4.55	0.86
Contribution of Line *Tester (%)	-	50.14	50.27	20.52

***Significant@P=0.001; **Significant@P=0.01; *Significant@P=0.05

TABLE 3

Estimates of tester-wise TC hybrid mean, variance and GCA effects for fruit yield plant^{-1} and its component traits

Testers	Traits	Mean	Variance	gca effects
83-016	Fruit yield plant^{-1} (kg)	01.01	0.42	0.07 ***
	Fruit diameter (cm)	03.43	0.11	0.12
	Fruit length (cm)	18.15	6.56	0.37
83-023	Fruit yield plant^{-1} (kg)	00.60	0.03	-0.16 ***
	Fruit diameter (cm)	03.43	0.08	-0.03
	Fruit length (cm)	16.58	1.81	-0.03
83-028	Fruit yield plant^{-1} (kg)	00.90	0.12	-0.12 ***
	Fruit diameter (cm)	03.63	0.13	-0.11
	Fruit length (cm)	21.83	0.43	-0.06
83-030	Fruit yield plant^{-1} (kg)	01.24	0.26	-0.01
	Fruit diameter (cm)	03.23	0.05	-0.03
	Fruit length (cm)	21.58	0.82	0.15
83-031	Fruit yield plant^{-1} (kg)	01.35	0.27	0.21 ***
	Fruit diameter (cm)	02.95	0.05	0.05
	Fruit length (cm)	15.78	0.46	-0.43
Bartlett's	Fruit yield plant^{-1} (kg)	-	159.36 **	-
x^2 statistic	Fruit diameter (cm)	-	75.79 **	-
	Fruit length (cm)	-	170.40 **	-

***Significant@P=0.001

suitable one as it most discriminated the ABLs for all the three traits. The greater discriminating ability of the tester, 83-016 could be attributed to presence of high frequency of unfavourable recessive alleles at loci controlling all the three traits for which ABLs are likely to possess high frequency of favourable dominant alleles. This is because, unfavourable recessive alleles present in the testers cannot mask the effect of favourable dominant alleles present in ABLs being tested, thereby maximizing the variance of TC hybrids (Bernardo, 2023). Castellanos *et al.* (1998) have also identified the most suitable testers based on the criterion of their ability to discriminate the test inbred lines for grain yield in maize.

Desirable Testers Based on High Trait Mean

The TC hybrid mean is a function of frequencies of alleles at loci controlling the target traits in the ABLs as well as in the testers. Hence, different testers used to evaluate same ABLs lead to different TC hybrid mean. In the present study, while the testers 83-031 and 83-030 produced TC hybrids with relatively greater mean for fruit yield plant^{-1}, the tester 83-028, closely followed by 83-016 and 83-030 produced TC hybrids with larger sized longer fruits than other testers (Fig. 1; Table 3). These testers namely 83-030 and 83031 for fruit yield plant^{-1} and 83-028 for size and length of the fruits are there fore could be considered as one of the parents of heterotic hybrid cultivars. This is because, new single cross hybrids are most often crosses between new inbred lines and an existing elite inbred line rather than crosses between two new inbred lines. The best inbred line identified based on the TC evaluation would be one parent and the elite tester itself would be another parent of the new hybrid.

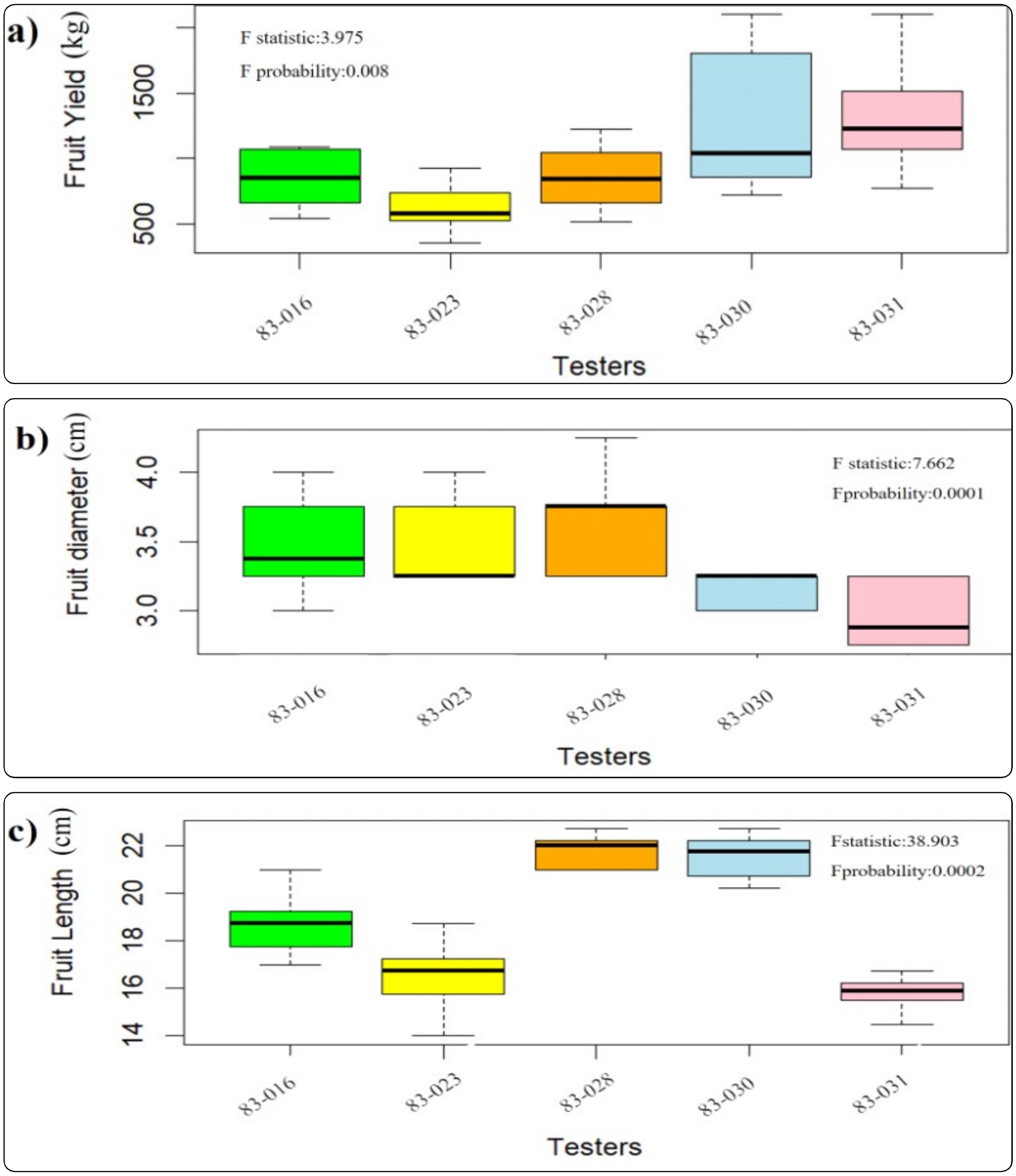

Fig. 1 : Box-Whisker plots showing comparative performance of five testers for (a) fruit yield plant^{-1} (b) fruit diameter and (c) fruit length

Desirable Testers Based on GCA

Producing testcross seeds is logistically difficult if each BP differs in the testers used. Consequently, only a few inbred lines typically serve as testers for each BP. As GCA indicate the worth of an inbred as a parent of multiple hybrids, estimates of GCA are useful for choosing a few key inbred lines to use as testers (Bernardo, 2023). In the present study, the tester 83-031 with greater magnitude of positive GCA effects could be considered as suitable one for fruit yield plant^{-1} and 83-016 for fruit length (Table 3). However, based on GCA criterion, none of the testers found suitable for fruit diameter.

General Combining Ability (GCA) of ABLs

The combining ability of an inbred line is the predicted performance of its progeny and hence it could be used as a reliable criterion to determine its commercial potential in hybrid combinations. The main advantage of combining ability is that it provides empirical summary of complex observations and a reasonable basis for assessing breeding value of parental lines and forecasting the performance of crosses without the complications of genetical assumptions (Bernardo 2020 and Bernardo, 2023). Being based on first degree statistics, combining ability effects are statistically robust and being genetically neutral, they are equally applicable to crops irrespective of their mode of pollination (Kundu *et al.,* 2022 and Zehra *et al*., 2023). In the present study, the ABLs differed significantly for their GCA effects for all the three QTs (Table 4). The wide range of estimates of GCA effects of the 10 ABLs suggested good ability of the testers to discriminate the lines for their GCA effects for fruit yield plant^{-1} and fruit length, but not for fruit diameter, which was attributable to the differences in frequencies of genes that are transmitted to the progeny with the additive effects (Falconer and Mackay, 1996). The differences in GCA effects of ABLs could also be attributable to presence of different additive effect alleles at loci controlling the three QTs among ABLs.

As expected, different ABLs were desirable general combiners for these traits (Table 4). In other words, no single ABL was a suitable general combiner for all the three QTs. For instance, ABLs such as 83-012 for fruit yield plant^{-1} and 83-010 for fruit diameter and fruit length were desirable good combiners. In addition, ABLs 83-020, 83-025 and ABTG-03 were good combiners for fruit yield plant^{-1}. The ABLs, 83-018, ABTG-08, 83-009, ABTG-09 and ABTG-10 require greater attention and need to be evaluated in a large scale to confirm their superiority for GCA effects (Ai-Zhi *et al*., 2012). These results are in conformity with those of Bajaj *et al*. (2007) in maize. It should however be noted that estimates of GCA effects of 10 ABLs lines are relative to and are dependent on particular set of lines and testers included in the study.

TABLE 4

Estimates of GCA effects of advanced breeding lines for quantitative traits

List of ABLs	Fruit yield plant^{-1} (kg)	Fruit diameter (cm)	Fruit length (cm)
ABTG-03	0.172 ***	-0.030	-0.130
ABTG-08	-0.190 ***	0.220	-1.130 **
ABTG-09	-0.415 ***	0.120	-2.580 ***
ABTG-10	-0.419 ***	0.070	-1.830 ***
83-009	-0.289 ***	0.220	2.970 ***
83-010	0.045 *	0.370 **	3.120 ***
83-012	0.628 ***	-0.180	2.970 ***
83-018	-0.188 ***	-0.030	2.620 ***
83-020	0.345 ***	-0.330 *	-3.030 ***
83-025	0.315 ***	-0.430 **	-2.980 ***
SEm±	0.02	0.13	0.40
CD @ P = 0.05	0.04	0.27	0.80

***Significant @ P=0.001; ** Significant @ P=0.01; * Significant @ P=0.05

Relationship of GCA Effects of Inbred Lines with their *per se* Performance

Significant positive but low magnitude of correlation between *per se* performance of the lines and their GCA effects for fruit yield plant^{-1} (Fig. 2), fruit diameter (Fig. 3) and fruit length (Fig. 4), indicated that it is not reliable to predict GCA effects of lines based on their *per se* performance for any of the traits. The poor correlation between *per se* performance and GCA effects of ABL could be attributable to different sets of genes controlling *per se* performance and GCA effects for target traits (Ai-Zhi and Zheng, 2012).

Relationship of Hybrid *per se* Performance with Sum of Parental GCA Effects

Relatively high magnitude of correlation between sum of the parental GCA effects with hybrid *per se* performance for all the three traits (Fig. 5, 6 & 7), suggested the possibility of reliable prediction of hybrid *per se* performance based on sum of GCA effects of their parents. Prediction of hybrid heterosis based on parental GCA effects would save substantial

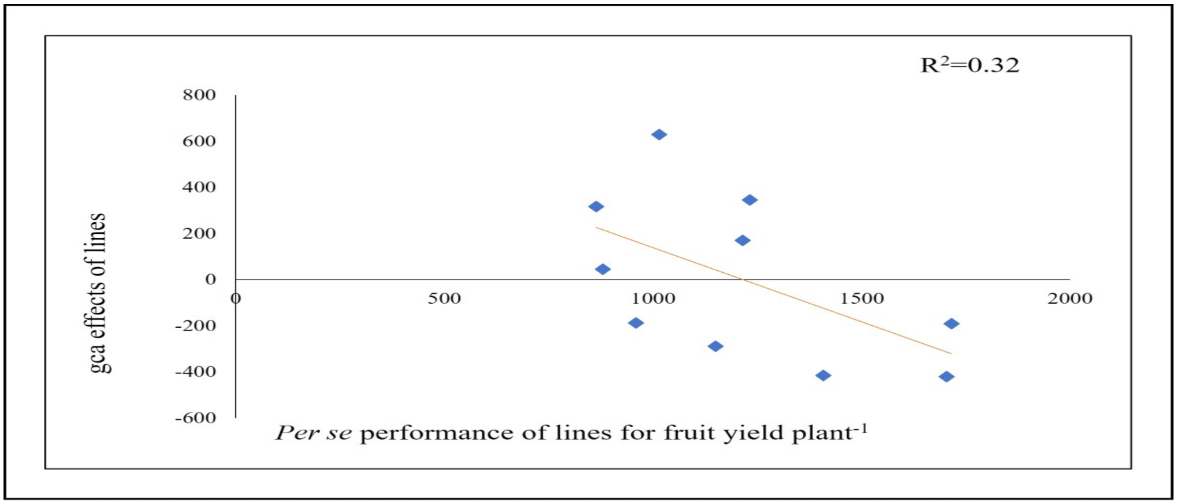

Fig. 2 : Correlation of *per se* performance of lines with their GCA effects for fruit yield plant^{-1}

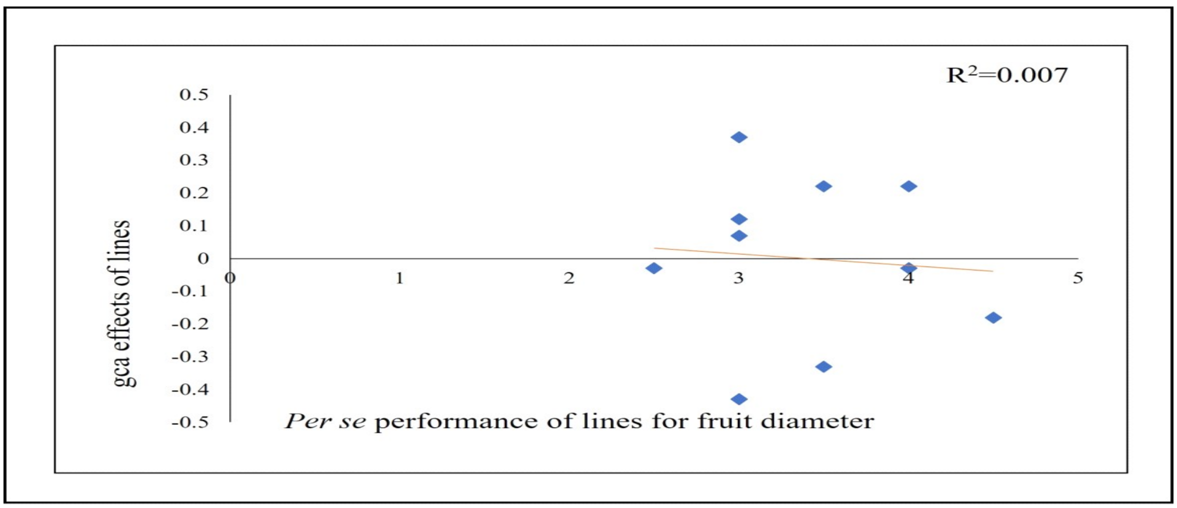

Fig. 3 : Correlation of *per se* performance of lines with their GCA effects for fruit diameter

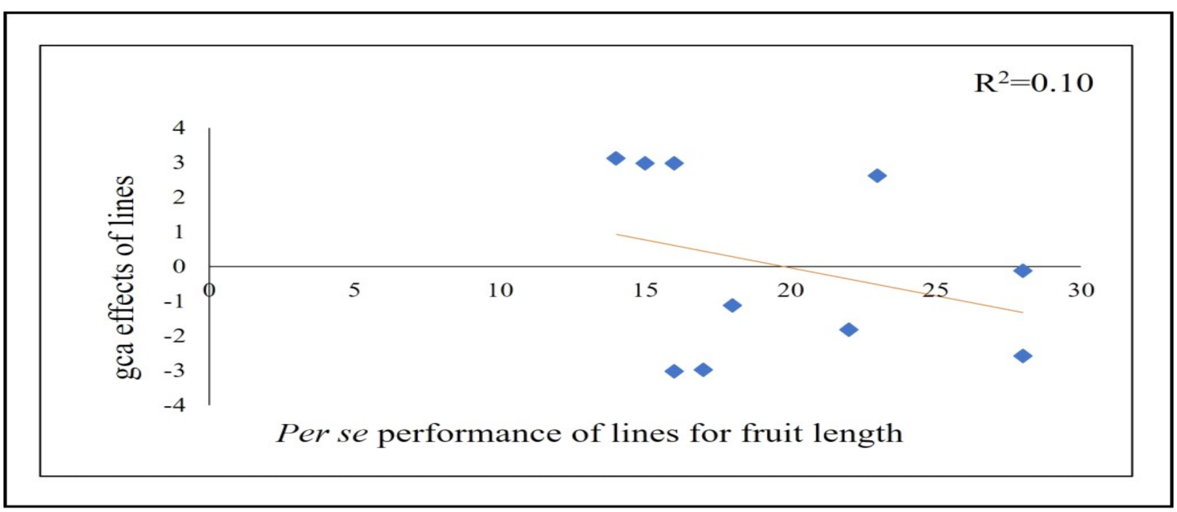

Fig. 4 : Correlation of *per se* performance of lines with their GCA effects for fruit length

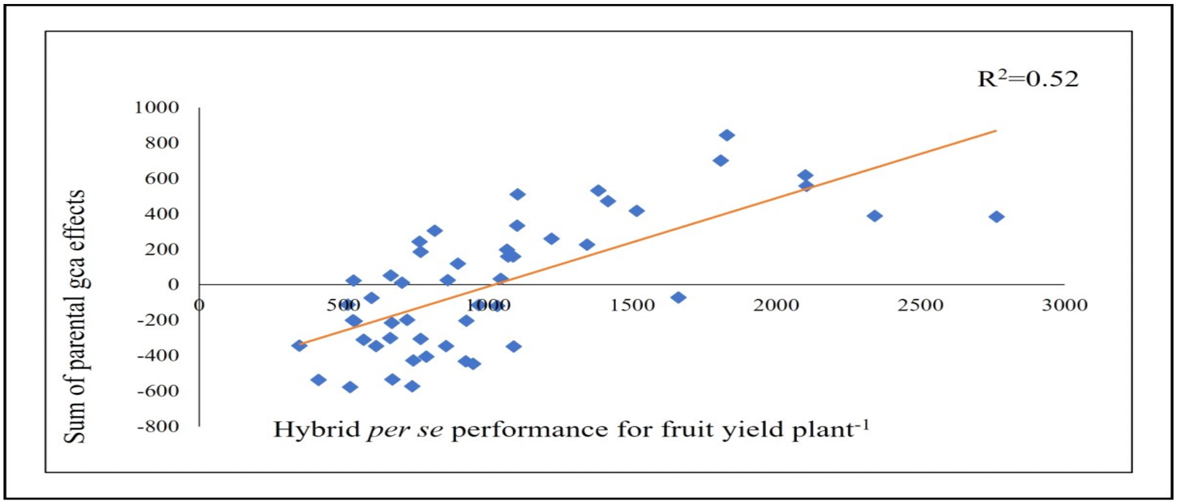

Fig. 5 : Correlation of hybrid *per se* performance with sum of parental GCA effects for fruit yield plant^{-1}

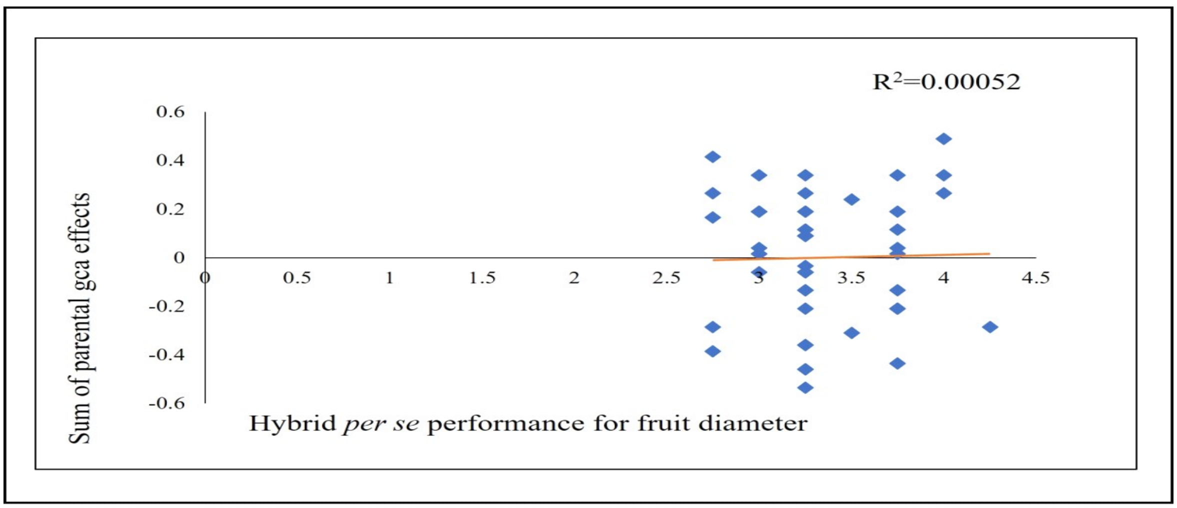

Fig. 6 : Correlation of hybrid *per se* performance with sum of parental GCA effects for fruit diameter

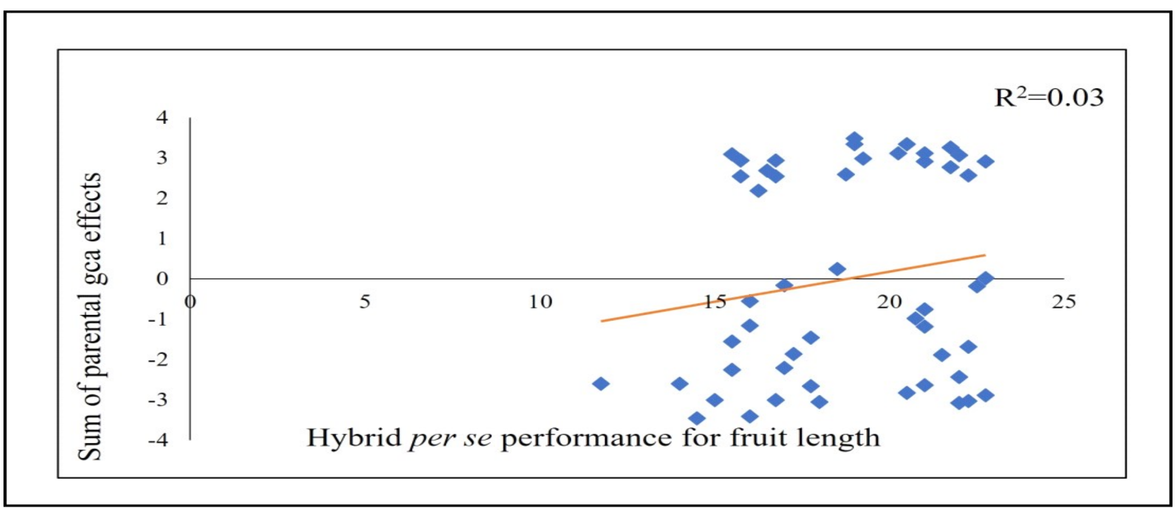

Fig. 7 : Correlation of hybrid *per se* performance with sum of parental GCA effects for fruit length

resources as it enables the evaluation of only a few hybrids that are predicted to be most promising ones. The utility of parental GCA effects for predicting hybrid *per se* performance has also been reported by Schrag *et al*. (2009), Sowmya & Gangappa (2018) and Sowjanya *et al*. (2019) in maize.

Selection of Best Heterotic Hybrids

Using Mayura as a standard check, the standard heterosis for each TC hybrid was calculated. On the basis of its standard heterosis, the top two TC hybrids *viz.*, ABTG-03×83-031 and 83-025×83-016 were chosen (Table 5). The heterotic potential of these hybrids needs to be confirmed based on large scale multi-location trial for their commercial exploitation. Further, the ABLs namely, ABTG-03, 83-031, 83-025 and 83-016 with significantly high hybrid potential could be recycled to derive new inbred lines with good prospects in hybrid combinations.

TABLE 5

Estimates of the best two hybrids with significantly higher standard heterosis for fruit yield plant^{-1}

Hybrids	Standard heterosis
ABTG-03×83-031	35.87
83-025×83-016	15.07
SEm±	0.07
CD @ P = 0.05	0.52

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