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Identification of the most suitable tester(s) from among parents of elite bitter gourd (*Momordica charantia* L.) single cross hybrids

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Abstract

In hybrid crop breeding programmes, the choice of tester is vital for efficient selection of experimental inbred lines for their potential in hybrid combinations. To identify such testers, 10 bitter gourd advanced breeding lines (ABLs) were crossed to five candidate testers. The resulting test cross (TC) hybrids were field-evaluated for fruit yield, fruit diameter, and fruit length in a two-replicated alpha-lattice design. Four criteria, namely (i) accuracy of ranking the ABLs, (ii) discrimination of the ABLs, (iii) high trait mean of TC hybrids and (iv) the high magnitude of gca effects, individually and in combination, were used to identify most suitable tester(s). The estimates of the correlation between ranks assigned to TC hybrids derived from each tester and those assigned to the TC hybrid mean of all five testers were used as statistics to quantify the accuracy of ranking ABLs by testers. The variance of TC hybrids derived from each tester was used as a statistic to quantify the ability of testers to discriminate the ABLs. The most suitable tester varied according to the criteria as well as the trait. Based on all four criteria, 83-031, 83-028 and 83-016 were identified as the most suitable testers for fruit yield, fruit diameter and fruit length. These testers are suggested for evaluating the performance of new experimental inbred lines developed from F2/backcross breeding populations derived from parents belonging to the same heterotic group and/or from any genetically variable source populations.

Key words: Advanced breeding lines, heterotic group, heterotic patterns, tester, test cross

Introduction

Bitter gourd is an economically and nutritionally vital cucurbitaceous vegetable, rich in carbohydrates, vitamins (ascorbic acid), proteins, and minerals, particularly Iron (Jat *et al.*, 2023; Srinivasulu *et al.*, 2024; Khatun *et al.*, 2024). Because of its several medical and therapeutic benefits, bitter gourd production is steadily increasing each year in Asian, European, and American nations (Shivanagoudra *et al.,* 2019). For the last two decades, bitter gourd has occupied a special position among vegetables due to its incomparable hypoglycemic and nutraceutical values (Tan *et al.,* 2016). Because of these features, bitter gourd is widely accepted as "vegetable insulin".

Despite being a significant global producer, India's average bitter gourd productivity is quite modest and has been steady for decades. Modest productivity of bitter gourd is attributed to the availability of a limited range of high-yielding cultivars suitable to a wide range of productive environments coupled with inadequate crop management and biotic and abiotic constraints (Rao *et al.*, 2021). Harnessing heterosis through the development and deployment of single cross hybrids (SCHs) is widely regarded as a cost-effective method to enhance the productivity of crops with a pollination system suitable for large-scale hybrid seed production. Bitter gourd is no exception to this.

Breeding single cross hybrids (SCHs) requires development of two good combining or nicking homozygous genotypes as parents. The homozygous genotypes for use as candidate hybrid parents are selected from segregating populations derived from two or more parents. The selection of such candidate hybrid parents should be based on their test cross (TC) hybrid performance, as the final cultivar option is F_1 hybrid. In this context, identification of the most convenient inbred line (s) referred to as a tester (s) assumes importance. There have been several studies on maize for the choice of tester (Kavya *et al.*, 2023). However, such studies in bitter gourd are seldom attempted. The present study envisages the identification of the most suitable tester (s) from among parents of elite SCHs for evaluation of the TC performance of advanced breeding lines (ABLs). The hypothesis of using the parents of elite SCHs as testers is that such parents produce TC hybrids with greater mean and variance and exhibit a high magnitude of gca effects and possess high frequency of favourable alleles that are likely to complement the low frequency of favourable alleles in ABLs and vice-versa (Reif *et al.*, 2005). These features of the parents of elite SCHs also likely to result in an accurate ranking of the ABLs for their TC hybrid potential.

This study was conducted to: (i) evaluate the accuracy of testers in ranking the ABLs, (ii) assess testers' ability to discriminate ABLs based on their TC hybrid potential, (iii) identify testers that yield TC hybrids with elevated trait means, (iv) identify testers exhibiting a greater magnitude of GCA effects in a favourable direction, and (v) determine the most suitable tester based on individual criteria and the aggregate of all four criteria.

Material and methods

Basic genetic material: The material for the study consisted of 10 ABLs selected from F_6 populations (Table 1). These 10 ABLs were selected based on their uniformity for plant type and flowering time. The material also consisted of five other genotypes, namely 83-016, 83-023, 83-028, 83-030 and 83-031, which were used as testers. These testers are the parents of elite single cross hybrids (Table 1).

Table 1. Fruit qualitative and quantitative traits of lines and testers used in the study

Sl. No.	Code	Fruit texture	Fruit colour	Fruit shape	Fruit length (cm)	Fruit diameter (cm)	Single fruit weight (g)
Lines							
01	A-BTG-003	SM	DG	SL	28	1.8- 2.0	130-140
02	A-BTG-008	SM	G	SPN	18	3.2-3.5	140-150
03	A-BTG-009	SP	LG	SL	28	2.5-2.8	170-180
04	A-BTG-010	SP	DG	SL	22	2.8-3.0	140-150
05	83-009	SP	G	SPN	16	4.0	75
06	83-010	SP	G	SPN	14	4.5	77
07	83-012	SP	G	SPN	9.5	2.5	20
08	83-018	SP	DG	SPN	23	4.5	145
09	83-020	SP	DG	SPN	14	4.5	63
10	83-025	SP	DG	SPN	17	3.0	72
Testers							
01	83-016	SM	DG	SLN	16	4.5	110
02	83-023	SM	LG	SPN	28	3.0	100
03	83-028	SM	G	SPN	20	4.0	96
04	80-030	SP	W	SLN	21	3.0	68
05	83-031	SP	W	SLN	20	3.0	63

SM: Smooth; SP: Spiny; DG: Dark Green; LG: Light Green; W: White; G: Green; SL: Slender long; SPN: Spindle; SLN: Slender

Development of experimental material: The seeds of ABLs and the five testers were sown in the nursery and 25-day-old healthy seedlings were transplanted to a crossing block. The ABLs were crossed to five testers to obtain 50 test cross (TC) hybrids (Table 2), which constituted the experimental genetic material for the study.

Field evaluation of experimental TC hybrids: The seeds collected from 50 TC hybrids were sown in the portrays in nursery at the research and developmental station, ORBI Seeds International Private Limited, Sadahalli, Bengaluru. The five healthy seedlings of each TC hybrid were transplanted in a single row of 3m length with a spacing of 1m between plants and 2m between rows in the experimental plot in alpha lattice design with two replications during 2023 rainy season. The recommended management practices were followed during the crop growth period to raise a healthy crop.

Sampling and data collection: The data were recorded on five 45-day-old plants of 50 TC hybrids in each replication for fruit diameter, fruit length and fruit yield. The marketable fruits were harvested in eight pickings from all five plants from each TC hybrid. These fruits were weighed and expressed as fruit yield plant^{-1}. In each TC hybrid, three fruits were selected randomly, which 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 3 fruits and expressed as average fruit diameter. For the same 3 fruits, the length was measured using a standard scale and expressed as the average fruit length.

Statistical analysis: Replication-wise, the means of TC hybrids for three traits were used for statistical analysis. Analysis of variance (ANOVA) for combining ability was performed by partitioning total variability among TC hybrids into sources attributable to hybrids, replications, lines, testers and line × tester interaction based on the linear model of line × tester mating design (Kempthorne, 1957). The analysis was implemented using 'INDOSTAT' software version 9.1. After ascertaining that TC hybrids of the five testers differ significantly from ANOVA, trait means of 10 TC hybrids derived from each tester was tested for their significance using one-way ANOVA using 'SPSS' software version 22.0. GCA effects of testers were estimated based on the same linear model of line × tester mating design.

The accuracy of testers to correctly rank the ABLs was quantified by correlation between ranks assigned to means of TC hybrids produced by each tester and to those assigned to means of TC hybrids produced by all the five testers. Analysis was implemented in 'R' studio version 4.3.3. Greater and significant rank correlation coefficients are interpreted as greater accuracy of the testers to rank the ABLs being tested correctly.

The efficiency of the testers to discriminate the ABLs was quantified as the 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 from each of the five testers was partitioned into sources attributable to hybrids, replications and residuals. Analysis was implemented using 'R' studio version 4.3.3. The 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 the number of replications.

Table 2. List of hybrids based on five testers used in the study

Hybrids based on 83-016 tester	Hybrids based on 83-023 tester	Hybrids based on 83-028 tester	Hybrids based on 83-030 tester	Hybrids based on 83-031 tester
ABTG-03×83-016	ABTG-03×83-023	ABTG-03×83-028	ABTG-03×83-030	ABTG-03×83-031
ABTG-08×83-016	ABTG-08×83-023	ABTG-08×83-028	ABTG-08×83-030	ABTG-08×83-031
ABTG-09×83-016	ABTG-09×83-023	ABTG-09×83-028	ABTG-09×83-030	ABTG-09×83-031
ABTG-10×83-016	ABTG-10×83-023	ABTG-10×83-028	ABTG-10×83-030	ABTG-10×83-031
83-009×83-016	83-009×83-023	83-009×83-028	83-009×83-030	83-009×83-031
83-010×83-016	83-010×83-023	83-010×83-028	83-010×83-030	83-010×83-031
83-012×83-016	83-012×83-023	83-012×83-028	83-012×83-030	83-012×83-031
83-018×83-016	83-018×83-023	83-018×83-028	83-018×83-030	83-018×83-031
83-020×83-016	83-020×83-023	83-020×83-028	83-020×83-030	83-020×83-031
83-025×83-016	83-025×83-023	83-025×83-028	83-025×83-030	83-025×83-031

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 the greater discriminating ability of the testers.

Results and discussion

Analysis of variance: The present study is aimed at identifying the most suitable tester that ranks accurately and discriminates ABLs with high magnitude of trait mean and gca effects. Addressing these objectives requires significant differences among the TC hybrids developed by crossing the ABLs with testers. The TC hybrids did differ significantly for all three traits, namely fruit yield plant^{-1}, fruit diameter and fruit length, as evident from significant mean squares attributable to hybrids (Table 3). Significant differences among the TC hybrids could be attributed to the differences in the frequency of alleles at loci controlling all three traits between ABLs and the testers. While significant mean squares of line effects could be attributed to additive effects and those of line × tester interaction (Table 3) effects could be attributed to non-additive effects resulting from the interaction between alleles at each locus (dominance) and those between loci (epistasis) controlling fruit yield and fruit diameter.

Table 3. Analysis of variance of TC hybrids derived from five testers for fruit yield and its component traits

Source of variation	Degrees of free-dom	Mean sum of squares		
		Fruit yield plant^{-1} (g)	Fruit diam-eter (cm)	Fruit length (cm)
Replications	01	6601.67	0.01	10.24***
Hybrids	49	543916.76***	0.26***	16.51***
Line effects	09	1279333.85**	0.65**	70.67***
Tester effects	04	451043.04	0.15	1.73
Line* Tester interac-tion effects	36	370381.79***	0.18*	4.61***
Error	49	1907.80	0.09	0.64
Contribution of Line (%)	-	43.20	45.18	78.63
Contribution of Tester (%)	-	6.77	4.55	0.86
Contribution of Line*Tester (%)	-	50.02	50.27	20.52

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

As 'F' test, as implemented in ANOVA is a rather less sensitive test, the contribution of additive effects of alleles from at least one tester cannot be ruled out despite non-significant mean squares attributable to testers for all the three traits (Table 3). The results of ANOVA indicate the possibility of identifying the most suitable tester (s).

Identification of most suitable tester(s): Hybrid breeding involves three inbred lines. Two of these, which belong to the same heterotic group (HG) are crossed to develop breeding populations (BPs) from which experimental inbred lines are derived. A third inbred line that belongs to opposite HG, designated as tester will be crossed to experimental inbred lines to evaluate their performance in hybrid combinations. In the present study, ABLs considered as experimental inbred lines were tested for their breeding potential based on their performance in TC hybrid combinations generated by crossing them with elite single cross hybrids' parents hypothesized as candidate testers. The ideal tester is the one which (i) accurately ranks the ABLs based on their TC hybrids performance, (ii) exhibits high efficiency in discriminating the ABLs, (iii) produces TC hybrids with greater mean performance for the target trait (s) and (iv) exhibits high magnitude of general combining ability (gca) in a desirable direction. We present and discuss the results based on these four criteria individually and in combination to identify the most suitable tester (s) for each of the three traits.

Accuracy of testers to rank ABLs: Given that true ranks of ABLs are not known *a priori*, estimates of the correlation between ranks assigned to TC hybrids derived from each tester and to those assigned to mean of TC hybrids derived from all the five testers has been suggested as the most suitable measure of the accuracy of ranking ABLs by testers (Castellanos *et al.*, 1998). The estimates of significant rank correlation coefficients suggested that testers, 83-030 and 83-031 accurately ranked the ABLs for their TC performance for fruit yield (Table 4). For fruit diameter, in addition to 83-030, three other testers, namely 83-016, 83-023 and 83-028, also accurately ranked the ABLs as evident from significant rank correlation coefficients. However, none of the testers accurately ranked the ABLs for fruit length. Based on the criterion of rank correlation coefficients between each tester and the mean of all the five testers, it is evident that the 83-030 could be regarded as the most suitable tester to accurately rank the ABLs for their TC hybrid performance for both fruit yield and fruit diameter. Abel and Pollak (1991) and Castellanos *et al.* (1998) have also reported non-significant rank correlation coefficients between pairs of testers and significant correlation coefficients between each tester and the mean of all the testers for grain yield in maize.

Table 4. Estimates of coefficients of rank correlation of test cross hybrids produced by each tester with mean of TC hybrids derived from all the five testers for fruit yield and its component traits

Mean of test cross hybrid derived from each tester	Mean of TC hybrids derived from all the five testers		
	Fruit yield	Fruit Diameter	Fruit length
83-016	0.36	0.70	-0.22
83-023	0.03	0.58*	0.31
83-028	-0.32	0.77**	0.15
83-030	0.82**	0.81**	-0.26
83-031	0.66*	0.34	0.07

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

Efficiency of testers to discriminate ABLs: The tester, 83-016 followed by 83-031 and 83-030, with significantly greater variance (Table 5) discriminated the ABLs better than the other two testers for fruit yield. For the other two traits, namely fruit diameter and fruit length, tester 83-016, with significantly greater variance, discriminated the ABLs better than the other three testers. Thus, based on the criterion of TC hybrids' variance, the tester 83-016 could be considered as the most 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 the presence of a high frequency of unfavourable recessive alleles at loci controlling all the three traits for which ABLs are likely to possess a 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.

Table 5. Analysis of variance (as per alpha lattice design) of tester-wise hybrids, and estimates of variances for fruit yield plant and its component traits

Source of variation	Degrees of freedom	Mean sum of squares														
		83-016			83-023			83-028			83-030			83-031		
		FY	FD	FL	FY	FD	FL	FY	FD	FL	FY	FD	FL	FY	FD	FL
Replication	01	182	0.31*	4.10	1307	0.11	5.51**	7792**	0.01	4.51**	3240	0.01	0.11	173	0.10	0.11
Hybrids.unadj	09	841428**	0.22**	13.11**	58012**	0.17	3.61**	237531**	0.26	0.86	514833**	0.10	1.64	536397**	0.10	0.93
Block/ Replication	01	8268	0.01	0.20	57	0.01	0.11	2069	0.31	0.61	268	0.11	0.31	2672	0.50*	2.11
Residual	08	5868	0.04	0.91	330	1.00	0.44	544	0.10	0.28	652	0.10	0.62	1862	0.06	0.77
Variance of test cross hybrids	-	417780.00	0.093	6.11	28841.00	0.04	1.59	118493.50	0.08	0.29	257090.50	0.001	0.51	267267.50	0.022	0.08

Bartlett's test (for examining homogeneity of variances) statistic (corrected chi-square statistic)

Fruit yield	Fruit diameter	Fruit length
159.36**	75.79**	170.40**

** Significant @ P=0.01; * Significant @ P=0.05; FY: Fruit yield; FD: Fruit diameter; FL: Fruit length

Ability of testers to produce TC hybrids with 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 the same ABLs lead to different TC hybrid mean. The tester (s) that accurately ranks the ABLs and efficiently discriminate the ABLs but not result in TC hybrids with high trait means cannot become a parent of heterotic hybrid cultivars that could be commercialized. Hence, such testers cannot be considered as ideal ones. In the present study, while the testers 83-031 and 83-030 produced TC hybrids with relatively greater mean for fruit yield plant^{-1}, tester 83028, closely followed by 83-016 and 83-030 produced TC hybrids with larger-sized longer fruits than other testers (Fig. 1). These testers namely 83-030 and 83031 for fruit yield and 83-028 for size and length of the fruits are therefore 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.

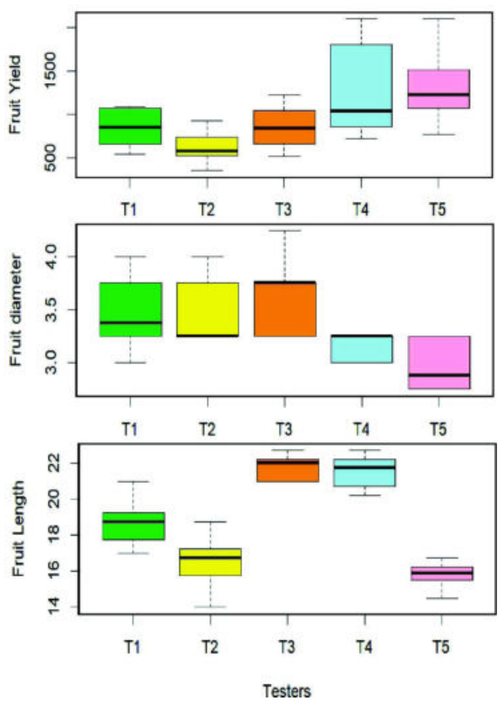

Fig. 1. Box-Whisker plots showing comparative performance of five testers for fruit yield and its component traits

Testers' general combining ability: 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 a greater magnitude of positive gca effects could be considered suitable for fruit yield and 83-016 for fruit length (Table 6). However, based on gca criterion, none of the testers found it suitable for fruit diameter.

Table 6. Estimates of GCA effects of testers for fruit yield and its component traits

Testers	Fruit yield plant^{-1} (kg)	Fruit diameter (cm)	Fruit length (cm)
83-016	73.21***	0.12	0.37*
83-023	-157.94***	-0.03	-0.03
83-028	-117.81***	-0.11	-0.06
83-030	-11.74	-0.03	0.15
83-031	214.28***	0.05	-0.43*

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

Identification of the most suitable tester (s) based on all the four criteria: The tester, 83-031, with relatively greater accuracy to rank and efficiently discriminate the ABLs and high trait mean and positive gca effects could be considered as the most suitable one for evaluating ABLs for their TC performance for fruit yield. The greater ability of the testers to discriminate the ABLs and to produce TC hybrids with high mean performance could be attributed to the dispersion of favourable dominant and unfavourable recessive alleles at fruit yield controlling loci between the testers and ABLs. As is true with respect to 83-031, the tester, 83-028 that produced TC hybrids with relatively greater mean and variance (barring gca effects) could be attributed to dispersion of favourable and unfavourable alleles at fruit diameter controlling loci between ABLs being tested and the testers. This tester therefore, could be regarded as the most suitable one for evaluating TC hybrid potential of ABLs for fruit diameter. With a little compromise in the accuracy of ranking the ABLs and mean of TC hybrids, the tester 83-016 could be considered the most suitable one for evaluating TC hybrid potential of ABLs for fruit length.

Implications of the results in bitter gourd hybrid breeding: The ABLs and the tester, 83-023, which produced TC hybrids with neither greater mean nor variance for any of the traits, could be included in one HG along with the ABLs. Three of the five testers, namely 83-016, 83-028, 83-030 and 83-031 could be grouped into opposite HG, especially for fruit yield. Classification of inbred lines into HGs streamlines hybrid breeding programme by dictating which pairs of inbred lines should be used as parents to be crossed to develop F_2 or backcross breeding populations (BPs) for identifying new inbred lines and which inbred line (s) should be used as tester(s) to evaluate the performance of new inbred lines derived from F_2/BC BPs. BPs in hybrid crops are typically developed by crossing two inbred lines from the same HG and using an inbred from an opposite HG as the tester. This is because advantage imparted by heterosis between inbred lines that belong to complementary HGs needs to be maintained during the development of new inbred lines and in the final hybrid produced. As the end product is a hybrid cultivar, the performance of new inbred lines needs to be assessed not in terms of their *per se* performance but in terms of their hybrid performance. The new inbred lines need to be evaluated for TC hybrid potential using 83-031 and 83-028, and 83-016 as testers for identifying new hybrid combinations for fruit yield, fruit diameter and fruit length, respectively.

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Conflict of interest: Authors of this manuscript declare that we have no conflict of interest.

Ethical approval: This manuscript does not contain studies performed by any of the authors involving humans or animals.

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