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Research Article

Line × Tester–Driven Assessment of Genetic Variability, Parental Contribution, and Hybrid Response for Yield Improvement in Bitter Gourd (*Momordica charantia* L.)

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ABSTRACT

Bitter gourd (*Momordica charantia* L.) is an economically important cucurbit in Pakistan, yet its productivity remains low due to limited availability of genetically improved hybrids. This study assessed genetic variability, combining ability, and heterosis in sixteen F_1 hybrids developed through line × tester mating design by crossing four female inbred lines (CKD-44, CKD-45, CKD-46, CKD-47) with four designated male testers (CKD-71, CKD-72, CKD-73, CKD-74). Significant differences among parents, hybrids, and line × tester interactions for all morphological and yield-related traits confirm substantial genetic variability. Highly heritable traits coupled with moderate to high genetic advance such as days to male and female flowering, number of primary branches, seeds per fruit, 100-seed weight, and yield per plant indicated the involvement of both additive and non-additive gene actions. Lines CKD-47, CKD-44, and CKD-46 exhibited superior general combining ability (GCA), while testers CKD-71, CKD-74, and CKD-73 also demonstrated favorable GCA for earliness and yield traits. The hybrids CKD-4572, CKD-4574, and CKD-4771 showed the highest specific combining ability (SCA), particularly for days to female flowering, primary branches, and days to maturity. Strong positive SCA effects in hybrids CKD-4474, CKD-4573, CKD-4671, and CKD-4774 translated into significant yield enhancement. Remarkable heterosis was observed in CKD-4473, CKD-4573, and CKD-4772, with yield heterosis peaking in CKD-4772 (90.91%) and CKD-4773 (93.94%), highlighting their commercial potential. Overall, both additive and non-additive genetic effects govern key traits. The parents CKD-44, CKD-46, CKD-47, CKD-71, CKD-73, and CKD-74, along with promising hybrids CKD-4474, CKD-4573, CKD-4671, and CKD-4774, are recommended for future bitter gourd breeding programs and multi-location evaluation.

Keywords: Bitter gourd, Line × tester analysis, Genetic variability, Combining ability (GCA and SCA), Heterosis, Hybrid breeding, yield improvement.

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INTRODUCTION

Bitter gourd (*Momordica charantia* L.) also known as bitter squash or bitter melon is a popular vegetable in the Indo-Pak subcontinent (Yaldiz *et al.*, 2025). It is a diploid type of vegetable with chromosome number 2n=2x=22. The species *Momordica dioica*, *Momordica tuberosa*, and *Momordica balsamina* L. are cultivated for their tasty fruits (Balsam apple). Its excellent nutritious value places it at the top among cucurbits with considerable amount of iron and vitamin C content. Due to its short lifespan and increasing consumer demand, it has earned the position of a cash crop.

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However, *M. charantia* is utilized as a vegetable, an ornamental plant, or an ayurvedic medicine. It is high in phenolic chemicals, antioxidants, and antimutagens (Naz, 2015). This versatile plant is cultivated globally for its culinary uses as well as for its traditional medicinal properties (Haque *et al.*, 2011).The plant is also known for its emmenagogue properties and used as an antiviral agent. It has also shown cholesterol-lowering, triglyceride-lowering, hypotensive, and insecticidal properties (Haque *et al.*, 2011). During 2021-2022, the total area under bitter gourd cultivation in Pakistan was 5,592 hectares, with a total yield of 45544 tonnes and with an average yield of 20.1t/h (Pakistan, 2022) as compared to 13-14 t/ha in India. India is the leading producer of bitter gourd, accounting for 31% of global production followed by China placing second position with a production share of 22%, while Pakistan holds the third position with 9% of the total production of bitter gourd worldwide (Khan *et al.*, 2024).

There are several factors responsible for the lower average yield of bitter gourd such as the non-availability of quality seeds of cultivars and hybrids with low genetic potential. By increasing vegetable production per unit area, it is possible to achieve high economic growth, thereby improving the socio-economic conditions of farmers (Naik, 2020). Plant breeders are interested in information on various qualitative and quantitative characteristics related to genetic variability, range, mean, heritability, and GA because they play an important role in the establishment of a successful crop improvement program (Pradhan *et al.*, 2020). Several biometrical parameters, including GCV and PCV, range, mean heritability, and genetic advance (GA), can be used to estimate the variability present in the genotype. Heritability is the inheritable trait that can be passed down from one generation to the next, whereas GA is the predicted gain of a specific character in the upcoming generation (Pradhan *et al.*, 2020).

In several vegetable crops, the value of heterosis breeding has been generally acknowledged. Heterosis refers to a phenomenon where an F_1 hybrid produced by mating two homozygous individuals with different genetic makeup, is more vigorous than its parents. The exploitation of heterosis is the way to produce quality seeds to enhance yield (Masud *et al.*, 2021). Through heterosis breeding breeders can boost yield and other economic attributes, which are effective for high output, uniformity, quality, and earliness (Naik, 2020). The GCA effects aid in choosing superior parents, while the SCA effects aid in choosing superior hybrids (Naik, 2020). This study exploited the phenomenon of heterosis to produce high-yielding bitter gourd hybrids to Morphological characterization of parental lines, to develop the crosses through line × tester mating design, Evaluation of crosses through combining ability and heterosis.

MATERIALS AND METHODS

Experimental Description

The present investigation was conducted at the University of Agriculture Faisalabad, Constituent College Depalpur, Okara, during the spring cropping season (February–June 2023) under open-field conditions. The experimental material comprised four female lines (CKD-44, CKD-45, CKD-46, and CKD-47), selected primarily for superior fruit shape attributes, and four male testers (CKD-71, CKD-72, CKD-73, and CKD-74), recognized for their high yield potential. A line × tester mating design was employed to generate sixteen F_1 hybrids. These hybrids, along with their respective parental lines and two commercial hybrid checks (Palee and Kiran), were evaluated to estimate genetic variability, combining ability, and heterosis in bitter gourd (*Momordica charantia* L.).

Nursery Raising and Transplantation

Seeds of parental lines, hybrids, and commercial checks were sown in 128-cell plastic trays filled with a peat moss and perlite mixture under protected nursery conditions. Seeds were placed at a depth of 1–2 cm and irrigated regularly to maintain optimal moisture levels. Trays were maintained under suitable temperature conditions to ensure uniform germination. Standard plant protection measures were adopted to minimize insect and disease incidence prior to transplanting.

Experimental Design and Field Layout

The experiment was conducted in a Randomized Complete Block Design (RCBD) with three replications. All genotypes, including parents, hybrids, and standard checks, were randomly assigned within each replication. Standard agronomic practices, including irrigation, fertilization, weed management, and plant protection measures, were uniformly applied throughout the experimental period to minimize environmental variation.

The following traits were recorded: days to male flowering, days to female flowering, days to maturity, vine length, number of primary branches per plant, fruit length, fruit diameter, number of fruits per plant, seeds per fruit, 100-seed weight, and yield per plant.

Statistical Analysis

The recorded data were subjected to analysis of variance (ANOVA) using the TNAUSTAT statistical package to determine the significance of genotypic differences. General and specific combining ability effects and heterosis estimates were computed to assess the magnitude and nature of genetic effects among hybrids following the methodology described by Mudhalvan *et al.* (2021). Genetic variability parameters were estimated using R software as described by Louis *et al.* (2023).

RESULTS AND DISCUSSION

The analysis of variance (ANOVA) revealed highly significant differences among lines, testers, crosses, line × tester interactions, and line versus tester effects for most morphological and yield-related traits, including days to male flowering, days to female flowering, number of primary branches, fruit length, number of seeds per fruit, node-to-node distance, 100-seed weight, days to maturity, number of fruits per plant, and yield per plant. Leaf area did not show significant variation. In addition to comparisons among parents and F_1 hybrids, hybrid performance was evaluated relative to the commercial check hybrids (Palee and Kiran). Several crosses exhibited superior mean performance and significant standard heterosis over these checks, particularly for yield per plant and related components, indicating their potential for commercial hybrid development. The significant variation observed for most traits confirms the presence of substantial genetic diversity among the evaluated genotypes. Since the primary objective of this study was to identify high-yielding and early-maturing hybrids with improved fruit characteristics, particular emphasis was placed on yield per plant, days to flowering, number of fruits per plant, and associated yield components.

Mean Performance of Parents, Hybrids, and Standard Checks

The mean performance of parents, hybrids, standard checks, and the grand mean for different traits is presented in (Table 1). Among the hybrids, CKD-4773 recorded the highest mean values for days to first male flowering (Table 2.), days to first female flowering, and yield per plant, indicating relatively late flowering behavior combined with superior yield performance compared with parents and standard checks. CKD-4771 exhibited the highest mean value for number of primary branches. Parent CKD-46 showed superior performance for fruit diameter, whereas hybrid CKD-4572 recorded the highest mean values for number of seeds per fruit and 100-seed weight. Parent CKD-73 exhibited the maximum mean value for days to maturity, while CKD-47 recorded the highest number of fruits per plant. These differences reflect the diverse genetic potential of the parental lines and their hybrids, highlighting the effectiveness of the line × tester approach in generating superior combinations, as originally described by Kempthorne (1957) and widely applied in vegetable breeding programs.

Estimates of Genetic Variability Parameters

Estimates of genetic variability parameters (Table 3) revealed high genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) for days to male flowering and days to female flowering, indicating wide variability and strong genetic influence on these traits. These observations are in agreement with Singh *et al.* (2017), Talukder *et al.* (2018), Tiwari *et al.* (2018), Singh and Kanadasmy (2020), Sravani *et al.* (2024), and Hasan *et al.* (2025). In contrast, fruit length and days to maturity exhibited low GCV and PCV values, suggesting limited genetic variability and reduced effectiveness of direct selection for these traits, as also reported by Singh *et al.* (2017), Singh and Kandasamy (2020), and Sravani *et al.* (2024). The remaining traits showed moderate GCV and PCV values, indicating intermediate levels of genetic variability. High heritability coupled with low genetic advance as percent of mean was observed for days to male flowering, number of primary branches, fruit length, number of seeds per fruit, node-to-node distance, 100-seed weight, days to maturity, number of fruits per plant, and yield per plant. This combination suggests the predominance of non-additive gene action, indicating that phenotypic selection for these traits may be less effective in early generations. Similar trends have been reported by Singh *et al.* (2017), Talukder *et al.* (2018), Tiwari *et al.* (2018), Sravani *et al.* (2024), and Hasan *et al.* (2025). In contrast, days to female flowering exhibited high heritability (98.89%) coupled with moderate genetic advance as percent of mean (39.73%), suggesting the involvement of both additive and non-additive gene effects. Therefore, while selection may be effective, hybrid breeding strategies may further enhance improvement for this trait, in agreement with Talukder *et al.* (2018).

General Combining Ability (GCA)

General combining ability (GCA) effects of parents are presented in (Table 3). Among the lines, CKD-44 showed the highest positive GCA effect for leaf area (9.37), while CKD-47 exhibited significant positive GCA effects for days to male flowering (3.27), days to female flowering (4.84), and number of primary branches (4.19). Among testers, CKD-71 recorded significant positive GCA effects for days to male and female flowering (1.03–1.20), followed by CKD-72 for number of primary branches (2.42) and fruit length (0.74). These results indicate the predominance of additive gene effects in these parents and suggest their suitability as superior combiners for the respective traits. Similar findings have been reported by Thangamani *et al.* (2011), Singh *et al.* (2018), Kamble *et al.* (2018), and Patel and Mehta (2021), although they differ from Srikanth *et al.* (2020).

Table 1. Mean performance of parents (Lines and Testers) and commercial check for selected yield-related traits in bitter gourd.

Genotype	DMF	DFF	NOPB	NF	YPP
Parents (Lines)					
CKD-44	16.13d	28.57c	12.00c	17.67c	3.10b
CKD-45	24.43a	38.87a	17.13a	16.67c	1.93d
CKD-46	24.00a	33.77b	12.53c	25.00b	2.93c
CKD-47	17.90c	29.57c	14.57b	28.67a	3.30a
Parents (Testers)					
CKD-71	13.77e	10.43d	19.87ab	23.67b	4.03a
CKD-72	17.20cd	26.13c	12.53c	21.67b	3.80b
CKD-73	21.23b	32.80b	20.67a	19.00c	2.87c
CKD-74	16.67d	29.57c	21.13a	15.67d	2.20d
Commercial Check (Pooled Mean)					
Check	20.20b	33.71b	14.56b	17.16c	2.51c
Grand Mean	18.58	32.92	29.00	20.24	3.33
SE (±)	0.4402	0.5551	1.0175	0.6389	0.0845
CD (0.05)	0.8981	1.1324	2.0758	1.3033	0.1724
CV (%)	2.90	2.06	4.30	3.87	3.10

Means followed by the same letter within a column are not significantly different at $P \leq 0.05$ according to LSD test. Whereas, DMF = days to male flowering; DFF = days to female flowering; NOPB = number of primary branches; NF = number of fruits per plant; YPP = yield per plant (kg).

Table 2. Performance of selected best- and least-performing hybrids based on yield per plant (YPP).

Hybrid	DMF	DFF	NF	YPP
Best-performing hybrids				
CKD-4773	24.47 a	43.63 a	28.00 ab	4.27 a
CKD-4772	24.90 a	42.10 b	28.73 a	4.20 a
CKD-4573	16.33 c	28.43 d	19.87 d	3.93 b
CKD-4771	24.23 a	42.53 b	22.93 c	3.93 b
CKD-4572	19.67 b	32.13 c	21.27 cd	3.63 c
Least-performing hybrids				
CKD-4674	16.33 c	34.10 c	14.67 f	2.03 f
CKD-4574	24.43 a	38.87 b	15.33 f	2.43 e
CKD-4473	16.70 c	27.43 d	17.27 e	2.97 d

Means followed by the same letter within a column are not significantly different at $P \leq 0.05$ according to LSD test.

Note: Hybrids were ranked based on yield per plant (YPP). Complete performance data of all hybrids and parents for all traits are provided in Supplementary Table S1. Whereas, DMF = days to male flowering; DFF = days to female flowering; NF = number of fruits per plant; YPP = yield per plant (kg).

Specific Combining Ability (SCA)

Specific combining ability (SCA) effects of hybrids are presented in Table 4. The crosses CKD-4572, CKD-4574, and CKD-4771 exhibited high SCA effects for days to female flowering, number of primary branches, and days to maturity, respectively. Notably, hybrids CKD-4474, CKD-4573, CKD-4671, and CKD-4774 exhibited significant positive SCA effects for yield per plant and also outperformed the commercial check hybrids (Palee and Kiran), indicating their suitability for commercial hybrid development.

These results highlight the importance of non-additive gene action in the expression of yield-related traits and are consistent with findings reported by Chandan *et al.* (2019), Jadav and Sapovadiya (2018), and Singh *et al.* (2018). These hybrids are therefore considered promising candidates for heterosis breeding.

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Table 2. Estimates of genetic variability parameters for yield and component traits.

Trait	GCV (%)	PCV (%)	Heritability	GAM (%)
DMF	22.02	22.21	98.30	44.97
DFF	19.43	19.53	98.89	39.79
NOPB	12.29	13.02	89.06	23.88
FL	4.73	5.71	68.71	8.08
FD	7.50	11.12	45.55	10.43
NF	19.80	20.17	96.33	40.03
YPP	18.38	18.59	97.76	37.44

Note: Complete variability parameters including ECV and GA are provided in Supplementary Table S2. Whereas, DMF = days to male flowering; DFF = days to female flowering; NOPB = number of primary branches; FL = fruit length (cm); FD = fruit diameter (cm); NF = number of fruits per plant; 100SW = 100-seed weight (g); YPP = yield per plant (kg).

Table 3. General combining ability (GCA) effects of hybrids for major yield traits.

Cross	NOPB	NF	100SW	YPP
CKD-4572	1.93*	0.31	2.08**	0.07
CKD-4771	1.89*	-2.17**	-2.25**	-0.24**
CKD-4671	1.89*	0.02	2.62**	0.37**
CKD-4574	2.17**	-1.56**	-0.18	-0.45**

Significant at P ≤ 0.05; ** significant at P ≤ 0.01. Full GCA estimates are given in Supplementary figure 1. Whereas, NOPB = number of primary branches; NF = number of fruits per plant; 100SW = 100-seed weight (g); YPP = yield per plant (kg).

Table 4. Specific combining ability (SCA) effects for yield-related traits.

Cross	NND	100SW	NF	YPP
CKD-4474	0.71*	1.70**	3.08**	0.51**
CKD-4572	0.42	2.08**	0.31	0.07
CKD-4671	1.28**	2.62**	0.02	0.37**
CKD-4774	-0.55	0.21	-0.52	0.22**

Full SCA estimates are given in Supplementary Table S3. Whereas, NND = node-to-node distance; NF = number of fruits per plant; 100SW = 100-seed weight (g); YPP = yield per plant (kg).

Table 5. Estimates of Heterosis (%) for Yield per Plant (YPP) in Selected Superior Hybrids.

Cross	Mid-Parent (%)	Better Parent (%)	Standard Heterosis (%)
CKD-4474	15.72**	-1.08NS	39.39**
CKD-4573	63.89**	37.21**	78.79**
CKD-4671	-4.31*	-17.36**	51.52**
CKD-4774	35.76**	13.13**	69.70**
CKD-4473	-0.56NS	-4.30NS	34.85**
CKD-4772	18.31**	10.53**	90.91**
CKD-4773	38.38**	29.29**	93.94**

Significance level at 0.05=*, Significance level at 0.01 = **, NS = Non-significant, Full SCA estimates are given in Supplementary Table S4-S6.

Estimates of Heterosis

The magnitude of heterosis over mid-parent (MP), better parent (BP), and standard check for yield per plant (YPP) is presented in (Tables 5). Considerable heterotic responses were observed across various assessed traits. For days to male flowering, maximum heterosis was recorded in CKD-4771 over MP, CKD-4471 over BP and CKD-4772 over the standard check. For days to female flowering, CKD-4771 showed maximum MP and BP heterosis, whereas CKD-

4773 recorded maximum standard heterosis. Similar heterotic trends have been reported by Podder *et al.* (2010), Abbasi *et al.* (2023), and Kundu *et al.* (2022). Negative heterosis observed for fruit length, fruit diameter, leaf area, and node-to-node distance indicates compact plant architecture, which may be desirable for improved resource-use efficiency and crop management, as reported by Quamruzzaman *et al.* (2009), Podder *et al.* (2010), Jadav and Sapovadiya (2018) Elshokri *et al.* (2022), , and. For yield-related traits, significant positive heterosis was observed for number of fruits per plant and yield per plant, particularly in hybrids CKD-4473, CKD-4573, and CKD-4772, confirming strong hybrid vigor, as also reported by Hannan *et al.* (2007), Ene *et al.* (2019), and Kundu *et al.* (2022).

Hybrids CKD-4474, CKD-4572, CKD-4573, CKD-4574, CKD-4671, CKD-4771, and CKD-4774 exhibited significant SCA effects and high heterosis and are therefore recommended for heterosis breeding. Moreover, hybrids such as CKD-4774, which exhibited non-significant SCA effects but were derived from parents with significant GCA effects, may serve as suitable candidates for recombination breeding using the pedigree method to identify superior segregants in subsequent generations.

CONCLUSION

The present investigation revealed substantial genetic variability among bitter gourd genotypes for key morphological and yield-related traits, confirming the suitability of the line × tester design for identifying superior parents and hybrid combinations. Significant variation among lines, testers, and their interactions indicated the association of both additive and non-additive gene actions in the inheritance of important economic traits.

Lines CKD-44, CKD-46, and CKD-47, along with testers CKD-71, CKD-73, and CKD-74, exhibited superior general combining ability particularly for yield per plant, days to flowering, and number of fruits per plant. Hybrids CKD-4474, CKD-4573, CKD-4671, and CKD-4774 demonstrated strong specific combining ability effects and significant standard heterosis for yield per plant compared with commercial checks (Palee and Kiran), indicating their potential for commercial hybrid development. The coexistence of additive and non-additive genetic effects suggests that both selection and heterosis breeding strategies can be effectively utilized for bitter gourd improvement. However, further multi-location evaluation is necessary before recommending these hybrids for large-scale cultivation.

AUTHOR CONTRIBUTIONS

Ghulam Murtaza: Writing - original draft, data analysis & formal analysis. Muhammad Qasim and Muhammad Talal Ahmad: Writing - review & editing. Waqar Ahmad: Resources (provided the germplasm). Sidra Iqbal: Validation, Visualization. Muhammad Rizwan Shafiq: Conceptualization, Methodology, Supervision, Resources, Validation.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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