



RESEARCH ARTICLE

# Harnessing hybrid vigor: Heterosis and combining ability analysis for enhanced yield in American cotton (*Gossypium hirsutum* L.)

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## Abstract

Harnessing heterosis is an effective strategy to improve cotton yield and fiber quality. The present investigation was undertaken to evaluate the magnitude of heterosis and combining ability for yield and fiber-related traits in cotton. A line × tester mating design involving six lines and two testers generated twelve F<sub>1</sub> hybrids, along with their parents and two commercial checks, were evaluated in a randomised block design with two replications at the Regional Agricultural Research Station (RARS), Warangal, Telangana, during Kharif 2024-2025. Data on yield and fiber quality parameters were analyzed using analysis of variance and heterosis and their effects were estimated. WGCV-372 emerged as the best general combiner (general combining ability (GCA) effect) for net plot yield/kg and boll weight. WGCV-372 exhibited positive GCA for boll weight, lint index, seed index and yield, indicating multi-trait superiority. The hybrids WGCV Bt-60 × PKV 081 Bt, WGCV Bt-60 × WGCV Bt-108 and WGCV-252 × PKV 081 Bt show strong heterosis improvement in yield, plant height, boll weight and fiber quality. The cross WGCV-252 × PKV 081 Bt showing strongest heterosis for boll weight with 39.1 % over mid-parent, 29.9 % over better parent and a 12.2 % increase over RCH 929 (check), indicating its potential to improve fiber yield components. WGCV Bt-60 × WGCV Bt-108 showed significant positive specific combining ability (SCA) for plant height, number of bolls per plant and net plot yield/kg. WGCV-413 × PKV 081 Bt exhibited positive SCA for plant height, monopodia, sympodia and number of bolls per plant. WGCV-252 × WGCV Bt-108 showed a significant positive SCA for lint index and ginning outturn (GOT %).

**Keywords:** combining ability; cotton; heterosis; yield

## Introduction

Cotton (*Gossypium* spp.), a cornerstone of global agriculture and industry, stands as the world's most widely used natural fiber and a leading non-food cash crop (1). Cotton cultivation shows significant global and regional variations in area, production and productivity. Globally, cotton production is projected to decline by 3 % to 117.8 million bales with a yield forecast of 830 kg per hectare, despite increased harvested area (2). Major production decreases are expected in China, India and Australia, while the United States, Brazil and Pakistan anticipate gains (2). In India, cotton cultivation during 2024-25 experienced an 8.7 % reduction in area from 123.70 to 112.94 lakh hectares compared to 2023-24. Production declined from 325.22 to 306.92 lakh bales (2). Telangana maintained its

position as the third-largest cotton-producing state in India during 2024-25 with 17.70 lakh hectares under cultivation, yielding 55.50 lakh bales (2).

The process of hybridization is indispensable for increasing genetic diversity within crop varieties, which is critical for improving essential traits such as yield, disease resistance and pest resistance. By mixing genetic material from diverse parents, breeders can create new genetic variations, thereby enabling plants to adapt more effectively to changing environmental conditions and potentially colonize new habitats. This method facilitates the combination of desirable characteristics from different parents into a single, improved offspring (3). To effectively evaluate and compare the performance of hybrids, plant breeders utilize various quantitative

measures of heterosis. These measures provide different perspectives on hybrid vigor, ranging from a general increase in performance to a direct assessment of commercial viability. Heterosis, or hybrid vigour, plays a pivotal role in genetic improvement across diverse crops (4). At the physiological level, heterosis is predominantly attributed to an increase in cell number rather than cell size (5). In plant breeding, the magnitude of heterotic expression in hybrids is typically quantified as the percentage increase or decrease over the mid-parent and better-parent values (6, 7). Two widely adopted metrics are used to describe hybrid performance. Mid-parent heterosis is the increase in yield or other character of the hybrid compared to the mean of the parents and is an estimate of the mean directional dominance, or potence, of alleles for a given character. Heterobeltiosis, on the other hand, measures the hybrid's advantage over the better-performing parent, indicating the presence of complementary dominant alleles dispersed between parents. This genetic complementation can either enhance or diminish the expression of the target trait, depending on the allelic interactions.

The success of hybrid breeding hinges on selecting and assessing high-quality parental lines. These lines can be assessed using genetic relatedness, heterosis and combining ability (8). A fundamental prerequisite for exploiting heterosis and accelerating genetic gain is the availability of sufficient genetic variability within breeding populations (9, 10). Quantifying interrelationships among yield-contributing traits enables breeders to apply indirect selection for complex quantitative characters, thereby improving selection efficiency (11). Comprehensive genetic diversity assessments support strategic parental selection and the formation of heterotic groups, which are critical for maximizing hybrid performance (12). A fundamental prerequisite for effective breeding programs is a comprehensive understanding of the genetic architecture underlying key agronomic and fiber traits. Combining ability analysis, encompassing general combining ability (GCA) and specific combining ability (SCA), provides invaluable insights for identifying elite parents and promising hybrid combinations (13). General combining ability quantifies the average performance of a parental line in a series of crosses, primarily reflecting additive gene action, which is fixable through selection (14). Conversely, SCA denotes the performance of specific cross combinations that deviate from expectations based on parental GCA, thereby indicating the presence of non-additive gene action, such as dominance and epistasis, which are exploitable in hybrid breeding (14). In view of the need to enhance cotton yield, this study aims to evaluate parental lines to determine heterosis and combining ability for diverse plant characteristics in cotton. Therefore, the present investigation was undertaken to assess heterosis and combining ability among six lines and two testers in American cotton for yield and its components. Therefore, the present investigation was undertaken to assess heterosis and combining ability among six lines and two testers in American cotton for yield and its components.

## Materials and Methods

The present investigation was conducted at the Regional Agricultural Research Station (RARS), Warangal, under Professor Jayashankar Telangana State Agricultural University (PJTAU), Telangana, India. The objective was to evaluate heterosis and combining ability in cotton using six lines (Co-17, WGCV-252, WGCV-413, WGCV-419, WGCV Bt-60 and WGCV-372) and two testers (WGCV

Bt-108 and PKV 081 Bt). These were crossed in an L × T mating design during Kharif 2023-2024, resulting in 12 F<sub>1</sub> hybrids. The evaluation was conducted during Kharif 2024-25, including the 12 F<sub>1</sub> hybrids, 8 parents and two commercial hybrids. As detailed in Table 1, the trial was laid out in a randomized block design (RBD) with two replications. Each entry (lines, testers, hybrids and checks) was sown in two rows per replication, with each row consisting of 40 plants and a plot size of 10.8 sq.m. Adherence to recommended plant spacing and crucial agronomic practices such as irrigation, fertilizer application and pest management were maintained throughout the cropping season to ensure optimal crop growth and performance.

The subsequent traits served as the basis for assessing heterosis and combining ability. Data were recorded from five randomly selected healthy and representative plants from each replication, except for net plot yield/kg, which was recorded on a plot basis.

**Plant height (cm):** Plant height was expressed in centimetres by measuring the selected plant's main stem from the ground level to the apex of the main stem at maturity.

**Number of monopodia per plant:** Number of monopodia branches per plant was recorded by counting the number of monopodia (non-fruitlet branches) at harvest.

**Number of sympodia per plant:** The number of sympodial branches per plant was recorded by counting the number of sympodia (fruiting branches) at harvest.

**Number of bolls per plant:** Number of bolls per plant was recorded by counting the number of bolls at the time of harvest.

**Boll weight (g):** Boll weight was expressed in grams by measuring the seed cotton taken from randomly collected 20 bolls.

**Seed index (g):** It is the absolute weight of 100 ginned seeds recorded in grams.

**Lint index (g):** It is the absolute weight of lint obtained from 100 seeds, including lint (kapas) recorded in grams.

**Ginning outturn (GOT) (%):** A random sample of 300 g seed cotton from each entry was ginned and the lint obtained was utilized for working out the ginning out-turn in the following manner.

$$\text{GOT (\%)} = (\text{Weight of lint (g)} / \text{Weight of seed cotton (g)}) \times 100 \quad (15)$$

**Net plot yield/kg:** The seed cotton harvested from the net plot area of each entry in a replication was weighed and expressed in kilograms per plot.

## Statistical analysis

The recorded observations were analyzed using the analysis of variance procedure as per the standard method (16). Estimates of heterosis were computed following the approach described earlier

**Table 1.** Details of lines, testers and F<sub>1</sub> hybrids

Lines	Testers	
	WGCV Bt-108	PKV 081 Bt
Co-17	Co-17 × WGCV Bt-108	Co-17 × PKV 081 Bt
WGCV-252	WGCV-252 × WGCV Bt-108	WGCV-252 × PKV 081 Bt
WGCV-413	WGCV-413 × WGCV Bt-108	WGCV-413 × PKV 081 Bt
WGCV-419	WGCV-419 × WGCV Bt-108	WGCV-419 × PKV 081 Bt
WGCV Bt-60	WGCV Bt-60 × WGCV Bt-108	WGCV Bt-60 × PKV 081 Bt
WGCV-372	WGCV-372 × WGCV Bt-108	WGCV-372 × PKV 081 Bt

(17). Combining ability effects were assessed in accordance with the methods outlined previously (18, 19). The data analysis was performed using Windostat Version 9.1 (Indostat Services).

## Results

### Analysis of variance

The mean square values from analysis of variance revealed that treatments, crosses, line effect, tester effect, line  $\times$  tester effect interactions were significant for most of the studied traits (Table 2 & 3). This included plant height, number of monopodia per plant, number of sympodia per plant, number of bolls per plant, boll weight, lint index, seed index, ginning outturn (%) and net plot yield/kg. Among these, the line effects were non-significant for traits like plant height, number of sympodia per plant, number of bolls per plant and ginning outturn. The tester effects remained non-significant for most traits such as number of monopodia per plant, number of bolls per plant, lint index, seed index, ginning outturn and net plot yield. Despite this, line  $\times$  tester interactions were highly significant for important traits including number of bolls per plant, boll weight, lint index, seed index, ginning outturn and net plot yield, indicating the presence of both additive and non-additive gene action across traits and the potential for exploiting hybrid vigour in cotton improvement.

### Assessment of mean performance of parents and $F_1$ hybrids

The average performance of the six lines, two testers and their 12  $F_1$  hybrids exhibited considerable variation in yield and yield-related traits, as detailed in Table 4 & 5. For plant height, the tallest parent was tester PKV 081 Bt (101.1 cm), while the shortest was line WGCV Bt-60 (73.1 cm). Among hybrids, WGCV-419  $\times$  WGCV Bt-108 was the tallest (102.8 cm) and WGCV-413  $\times$  WGCV Bt-108 shortest (80.6 cm). Number of bolls per plant, line WGCV-252 led parents (11.5) with line WGCV Bt-60 lowest (7.2), hybrid WGCV Bt-60  $\times$  PKV 081 Bt excelled (13.0), while WGCV-413  $\times$  PKV 081 Bt was lowest (9.0). For boll weight, tester WGCV Bt-108 topped parents (5.0 g) and hybrid WGCV-252  $\times$  PKV 081 Bt was highest (4.9 g) with WGCV Bt-60  $\times$  PKV 081 Bt and WGCV-372  $\times$  WGCV Bt-108 at the minimum (3.8 g). Ginning outturn was highest in parent line WGCV-252 (37.55 %) and lowest in line WGCV Bt-60 (28.78 %). Hybrid WGCV-419  $\times$  PKV 081 Bt performed best (36.39 %) and WGCV-372  $\times$  WGCV Bt-108 worst (29.85 %). Net

plot yield showed line WGCV-413 as the top parent (2.8 kg plot<sup>-1</sup>) and line WGCV Bt-60 lowest (1.51 kg plot<sup>-1</sup>), hybrid WGCV-252  $\times$  PKV 081 Bt achieved the maximum (3.4 kg plot<sup>-1</sup>), surpassing parents, while Co-17  $\times$  PKV 081 Bt was minimal (2.26 kg plot<sup>-1</sup>).

### Manifestation of heterosis in specific traits

Heterotic responses of the  $F_1$  hybrids in comparison to their mid-parent, better parent and standard checks (RCH 929 and Swift) across various agronomic characters are detailed in (Table 6-10). The cross WGCV Bt-60  $\times$  WGCV Bt-108 expressing strong positive heterosis over the mid-parent (21.95 %), better parent (33.79 %), RCH 929 (26.36 %) and Swift (8.55 %), while WGCV Bt-60  $\times$  PKV 081 Bt also showed notable increases over the mid-parent (8.27 %), better parent (29.00 %), RCH 929 (21.83 %) and Swift (4.66 %), indicating robust hybrid vigor and potential for enhancing plant stature. Conversely, WGCV-413  $\times$  WGCV Bt-108 demonstrated significant negative heterosis over the mid-parent (-8.04 %), better parent (-7.67 %) and Swift (-10.54 %), while WGCV-413  $\times$  PKV 081 Bt recorded -6.40 % over the mid-parent and -1.78 % relative to Swift, suggesting transgressive segregation for reduced plant height an important attribute for developing compact genotypes suitable for high-density planting and mechanized harvesting.

The cross WGCV Bt-60  $\times$  PKV 081 Bt, which exhibited the highest heterosis over the mid-parent (46.52 %) and marked increases over the better parent (23.02 %), with impressive superiority over the standard check Swift (30.40 %); however, its advantage over RCH 929 was comparatively modest (-3.41 %). Another high-performing cross, WGCV Bt-60  $\times$  WGCV Bt-108, displayed significant heterosis with 34.08 % over the mid-parent, 22.52 % over the better parent and solid increases relative to Swift (6.60 %), though it was less competitive versus RCH 929 (-21.04 %). Similarly, Co-17  $\times$  WGCV Bt-108 also showed strong heterotic effects, recording 29.44 % over the mid-parent and 27.95 % over the better parent, as well as a positive 11.32 % over Swift, but negative heterosis with respect to RCH 929 (-17.54 %).

For boll weight, notable heterotic effects were identified among the  $F_1$  hybrids with WGCV-252  $\times$  PKV 081 Bt emerging as a top performer, exhibiting substantial positive heterosis over the mid-parent (39.1 %), better parent (29.9 %) and standard check RCH 929 (12.2 %), though it showed a slight decrease relative to Swift (-1.3 %).

**Table 2.** Anova mean squares for nine yield and yield-related traits

Source	DF	Plant height	Number of monopodia per plant	Number of sympodia per plant	Number of bolls per plant
Replicates	1	0.033	1.245	6.881	0.006
treatments	21	124.742***	0.931*	4.292*	4.205***
Crosses	11	115.096***	1.296*	3.821*	3.451*
Line effect	5	36.206	2.414***	4.223	3.196
Tester effect	1	473.482***	0.082	9.127	1.955
Line $\times$ Tester effect	5	122.310	0.422	2.359	4.005**
Error	11	38.416	0.213	1.984	1.124

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

**Table 3.** Anova mean squares for nine yield and yield-related traits

Source	DF	Boll weight (g)	Lint index (g)	Seed index (g)	Ginning outturn (%)	Net plot yield / kg
Replicates	1	0.019	0.001	2.318	12.445*	0.031
treatments	21	0.435***	0.698***	2.110***	14.125***	0.553***
Crosses	11	0.539***	0.789***	3.154***	16.091***	0.581***
Line effect	5	0.504**	0.789**	5.412***	12.645***	0.865***
Tester effect	1	0.799**	0.032	0.92	3.604	0.015
Line $\times$ Tester effect	5	0.522**	0.948***	1.342**	22.035***	0.381***
Error	11	0.113	0.171	0.399	2.263	0.055

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

**Table 4.** Evaluation of mean values for nine yield and yield components in lines, testers and F<sub>1</sub> hybrids

Lines and testers	Plant height (cm)	Number of monopodia per plant	Number of sympodia per plant	Number of bolls per plant
<b>Lines</b>				
Co-17	88.30	0.0	13.8	8.5
WGCV-252	93.00	0.3	17.3	11.5
WGCV-413	88.00	1.4	13.5	10.6
WGCV-419	93.90	1.2	14.1	9.7
WGCV Bt-60	73.10	2.1	13.3	7.2
WGCV-372	100.50	2.4	15.4	9.2
<b>Testers</b>				
WGCV Bt-108	87.30	1.1	15.2	8.7
PKV 081 Bt	101.10	0.1	15.0	10.6
<b>Average</b>	90.7	1.1	14.7	9.5
<b>F<sub>1</sub> hybrids</b>				
Co-17 × WGCV Bt-108	98.00	0.2	15.3	11.1
Co-17 × PKV 081 Bt	92.20	0.6	17.2	9.1
WGCV-252 × WGCV Bt-108	89.40	0.9	13.9	11.2
WGCV-252 × PKV 081 Bt	96.70	1.8	13.4	10.6
WGCV-413 × WGCV Bt-108	80.60	0.5	16.0	9.2
WGCV-413 × PKV 081 Bt	88.50	1.3	13.7	9.0
WGCV-419 × WGCV Bt-108	102.80	0.0	13.1	10.5
WGCV-419 × PKV 081 Bt	102.70	0.8	17.4	10.3
WGCV Bt-60 × WGCV Bt-108	97.80	0.7	14.6	10.7
WGCV Bt-60 × PKV 081 Bt	94.30	1.6	15.3	13.0
WGCV-372 × WGCV Bt-108	89.50	0.6	14.4	10.9
WGCV-372 × PKV 081 Bt	98.40	0.3	14.9	9.1
<b>Average</b>	94.2	0.8	14.9	10.4
<b>LSD at 5 %</b>	13.06	1.14	2.32	2.47

**Table 5.** Evaluation of mean values for nine yield and yield components in lines, testers and F<sub>1</sub> hybrids

Lines and testers	Boll weight (g)	Lint index (g)	Seed index (g)	Ginning outturn (%)	Net plot yield/kg
<b>Lines</b>					
Co-17	4.3	4.8	8.4	36.24	2.22
WGCV-252	3.3	4.9	8.2	37.55	2.49
WGCV-413	4.1	5.1	8.4	37.46	2.80
WGCV-419	3.8	3.8	8.6	30.78	2.35
WGCV Bt-60	3.6	4.0	9.9	28.78	1.51
WGCV-372	4.0	5.2	9.5	35.36	2.29
<b>Testers</b>					
WGCV Bt-108	5.0	5.5	11.9	31.54	2.67
PKV 081 Bt	3.8	4.6	9.4	32.54	2.60
<b>Average</b>	4.0	4.7	9.3	33.8	2.4
<b>F<sub>1</sub> hybrids</b>					
Co-17 × WGCV Bt-108	4.5	5.3	11.4	31.63	3.26
Co-17 × PKV 081 Bt	4.0	6.2	11.0	36.06	2.26
WGCV-252 × WGCV Bt-108	4.4	5.0	9.6	34.24	3.22
WGCV-252 × PKV 081 Bt	4.9	5.1	10.6	32.29	3.40
WGCV-413 × WGCV Bt-108	4.6	4.5	10.4	30.26	2.65
WGCV-413 × PKV 081 Bt	4.6	5.3	10.5	33.47	2.53
WGCV-419 × WGCV Bt-108	4.2	5.4	9.8	35.66	2.81
WGCV-419 × PKV 081 Bt	3.9	5.2	9.0	36.39	2.59
WGCV Bt-60 × WGCV Bt-108	4.7	4.4	8.2	35.09	3.23
WGCV Bt-60 × PKV 081 Bt	3.8	4.3	9.2	31.92	3.34
WGCV-372 × WGCV Bt-108	3.9	4.0	9.5	29.85	2.81
WGCV-372 × PKV 081 Bt	4.4	5.2	9.2	36.10	2.52
<b>Average</b>	4.3	5.0	9.8	33.6	2.9
<b>LSD at 5 %</b>	0.72	0.90	1.55	3.30	0.60

WGCV-413 × PKV 081 Bt also delivered significant positive heterosis with 15.7 % over the mid-parent, 11.5 % over the better parent and a marginal gain over RCH 929 (3.9 %), despite a negative value compared to Swift (-8.6 %). In contrast, WGCV-372 × WGCV Bt-108 demonstrated pronounced negative heterosis with -12.8 % over the mid-parent, -21.5 % over the better parent and -10.8 and -21.5 % over RCH 929 and Swift, respectively.

For GOT, heterotic responses varied among the F<sub>1</sub> hybrids with WGCV-419 × PKV 081 Bt and WGCV-419 × WGCV Bt-108 showing the highest positive heterosis over the mid-parent at 14.9 % and 14.4 %, respectively along with improvements over the better parent (11.8 and 13.1 %) and slight positive gains relative to Swift (2.7 and 0.7 %). WGCV Bt-60 × WGCV Bt-108 also demonstrated notable positive heterosis: 16.3 % over the mid-parent and 11.2 % over the

better parent, though it showed small negative heterosis compared to both RCH 929 (-4.3 %) and Swift (-1.0 %). Conversely, some crosses, such as WGCV-413 × WGCV Bt-108 and WGCV-372 × WGCV Bt-108, exhibited substantial negative heterosis, with reductions of -12.3 and -10.8 % over mid-parent and even larger decreases compared to better parents and standard checks.

For net plot yield, hybrids WGCV Bt-60 × PKV 081 Bt and WGCV Bt-60 × WGCV Bt-108 exhibited the highest positive heterosis with 62.4 and 54.4 % increases over the mid-parent, respectively. Both hybrids also showed substantial heterosis over the better parent (28.3 and 20.9 %) and modest positive gains relative to Swift (3.9 and 0.5 %), although all hybrids showed negative heterosis compared to the higher-yielding RCH 929 check, with the best performing hybrids showing around -15 to -18 % reductions. Other



**Table 6.** Comparison of heterotic effects of F<sub>1</sub> hybrids over mid-parent, better parent and standard checks (RCH 929 and Swift) for plant height

F <sub>1</sub> hybrids	Line	Tester	Mid parent	F <sub>1</sub>	Percentage increase (+) or decrease (-) of F <sub>1</sub> over			
					Mid parent	Better parent	RCH 929	Swift
Co-17 × WGCV Bt-108	88.30	87.30	87.8	98.00	11.62	12.26	26.61	8.77
Co-17 × PKV 081 Bt	88.30	101.10	94.7	92.20	-2.64	4.42	19.12	2.33
WGCV-252 × WGCV Bt-108	93.00	87.30	90.2	89.40	-0.83	2.41	15.50	-0.78
WGCV-252 × PKV 081 Bt	93.00	101.10	97.1	96.70	-0.36	3.98	24.94	7.33
WGCV-413 × WGCV Bt-108	88.00	87.30	87.7	80.60	-8.04	-7.67	4.13	-10.54
WGCV-413 × PKV 081 Bt	88.00	101.10	94.6	88.50	-6.40	0.57	14.34	-1.78
WGCV-419 × WGCV Bt-108	93.90	87.30	90.6	102.80	13.47	17.75	32.82	14.10
WGCV-419 × PKV 081 Bt	93.90	101.10	97.5	102.70	5.33	9.37	32.69	13.98
WGCV Bt-60 × WGCV Bt-108	73.10	87.30	80.2	97.80	21.95	33.79	26.36	8.55
WGCV Bt-60 × PKV 081 Bt	73.10	101.10	87.1	94.30	8.27	29.00	21.83	4.66
WGCV-372 × WGCV Bt-108	100.50	87.30	93.9	89.50	-4.69	2.52	15.63	-0.67
WGCV-372 × PKV 081 Bt	100.50	101.10	100.8	98.40	-2.38	-2.09	27.13	9.21
Check-1 RCH 929	77.40							
Check-2 Swift	90.10							

**Table 7.** Comparison of heterotic effects of F<sub>1</sub> hybrids over mid-parent, better parent and standard checks (RCH 929 and Swift) for number of bolls per plant

F <sub>1</sub> hybrids	Line	Tester	Mid parent	F <sub>1</sub>	Percentage increase (+) or decrease (-) of F <sub>1</sub> over			
					Mid parent	Better parent	RCH 929	Swift
Co-17 × WGCV Bt-108	8.50	8.70	8.6	11.1	29.44	27.95	-17.54	11.32
Co-17 × PKV 081 Bt	8.50	10.60	9.6	9.1	-4.80	-14.23	-32.65	-9.08
WGCV-252 × WGCV Bt-108	11.50	8.70	10.1	11.2	10.55	-2.91	-17.29	11.66
WGCV-252 × PKV 081 Bt	11.50	10.60	11.1	10.6	-4.05	-7.81	-21.47	6.02
WGCV-413 × WGCV Bt-108	10.60	8.70	9.7	9.2	-4.18	-12.77	-31.51	-7.54
WGCV-413 × PKV 081 Bt	10.60	10.60	10.6	9.0	-15.35	-15.35	-33.54	-10.27
WGCV-419 × WGCV Bt-108	9.70	8.70	9.2	10.5	14.52	8.62	-21.95	5.36
WGCV-419 × PKV 081 Bt	9.70	10.60	10.2	10.3	1.55	-2.76	-23.65	3.08
WGCV Bt-60 × WGCV Bt-108	7.20	8.70	8.0	10.7	34.08	22.52	-21.04	6.60
WGCV Bt-60 × PKV 081 Bt	7.20	10.60	8.9	13.0	46.52	23.02	-3.41	30.40
WGCV-372 × WGCV Bt-108	9.20	8.70	9.0	10.9	22.28	18.96	-18.93	9.44
WGCV-372 × PKV 081 Bt	9.20	10.60	9.9	9.1	-8.09	-14.16	-32.60	-9.01
Check-1 RCH 929	13.5							
Check-2 Swift	10.0							

**Table 8.** Comparison of heterotic effects of F<sub>1</sub> hybrids over mid-parent, better parent and standard checks (RCH 929 and Swift) for boll weight

F <sub>1</sub> hybrids	Line	Tester	Mid parent	F <sub>1</sub>	Percentage increase (+) or decrease (-) of F <sub>1</sub> over			
					Mid parent	Better parent	RCH 929	Swift
Co-17 × WGCV Bt-108	4.3	5.0	4.7	4.5	-2.7	-9.5	2.8	-9.5
Co-17 × PKV 081 Bt	4.3	3.8	4.1	4.0	-0.8	-6.6	-8.7	-19.7
WGCV-252 × WGCV Bt-108	3.3	5.0	4.2	4.4	6.5	-11.6	0.5	-11.6
WGCV-252 × PKV 081 Bt	3.3	3.8	3.6	4.9	39.1	29.9	12.2	-1.3
WGCV-413 × WGCV Bt-108	4.1	5.0	4.6	4.6	0.2	-8.8	3.6	-8.8
WGCV-413 × PKV 081 Bt	4.1	3.8	4.0	4.6	15.7	11.5	3.9	-8.6
WGCV-419 × WGCV Bt-108	3.8	5.0	4.4	4.2	-5.3	-16.7	-5.3	-16.7
WGCV-419 × PKV 081 Bt	3.8	3.8	3.8	3.9	2.4	2.4	-11.5	-22.2
WGCV Bt-60 × WGCV Bt-108	3.6	5.0	4.3	4.7	9.5	-5.8	7.0	-5.8
WGCV Bt-60 × PKV 081 Bt	3.6	3.8	3.7	3.8	2.2	-0.5	-14.1	-24.4
WGCV-372 × WGCV Bt-108	4.0	5.0	4.5	3.9	-12.8	-21.5	-10.8	-21.5
WGCV-372 × PKV 081 Bt	4.0	3.8	3.9	4.4	13.3	10.5	0.5	-11.6
Check-1 RCH 929	4.4							
Check-2 Swift	5.0							

notable hybrids include Co-17 × WGCV Bt-108 and WGCV-252 × PKV 081 Bt, which recorded 33.4 % heterosis over mid-parent and above 20 % over better parent, but also exhibited negative heterosis compared to RCH 929.

The hybrids WGCV Bt-60 × PKV 081 Bt, WGCV Bt-60 × WGCV Bt-108 and WGCV-252 × PKV 081 Bt demonstrated significant heterosis and hybrid vigor for key yield and agronomic traits. WGCV Bt-60 × PKV 081 Bt showed the highest heterosis for net plot yield (62.4 % over mid-parent), strong plant height heterosis (46.52 %) and a notable boll weight advantage (39.1 %), indicating exceptional yield potential. WGCV Bt-60 × WGCV Bt-108 exhibited substantial heterosis for net plot yield (54.4 %), plant height (34.08 %) and ginning outturn (16.3 %), reflecting consistent improvement across multiple traits. WGCV-252 × PKV 081 Bt was a top performer for boll weight (39.1 %) and also demonstrated strong heterosis for net plot

yield (33.4 %), highlighting its value in enhancing both yield and boll quality. These hybrids represent promising candidates for cotton breeding programs focused on improving productivity and fiber traits.

#### General and specific combining ability effects analysis

Table 11-14 present the GCA and SCA effects for various agronomic traits of the parental lines, testers and their F<sub>1</sub> hybrids. The best general combiner for yield was WGCV-372, showing a highly significant positive GCA effect for net plot yield (0.718<sup>\*\*\*</sup>) and also for boll weight (0.529<sup>\*\*</sup>). WGCV Bt-60 was another strong general combiner with significant positive GCA for boll weight (0.122), lint index (0.767<sup>\*\*</sup>), seed index (1.429<sup>\*\*</sup>) and moderate improvement in net plot yield (0.173). Among testers, none showed significant effects for yield, but PKV 081 Bt exhibited the strongest positive GCA for

**Table 9.** Comparison of heterotic effects of F<sub>1</sub> hybrids over mid-parent, better parent and standard checks (RCH 929 and Swift) for ginning outturn

F <sub>1</sub> hybrids	Line	Tester	Mid parent	F <sub>1</sub>	Percentage increase (+) or decrease (-) of F <sub>1</sub> over			
					Mid parent	Better parent	RCH 929	Swift
Co-17 × WGCV Bt-108	36.24	31.54	33.9	31.63	-6.7	-12.7	-13.7	-10.7
Co-17 × PKV 081 Bt	36.24	32.54	34.4	36.06	4.8	-0.5	-1.7	1.8
WGCV-252 × WGCV Bt-108	37.55	31.54	34.5	34.24	-0.9	-8.8	-6.6	-3.3
WGCV-252 × PKV 081 Bt	37.55	32.54	35.0	32.29	-7.9	-14.0	-11.9	-8.9
WGCV-413 × WGCV Bt-108	37.46	31.54	34.5	30.26	-12.3	-19.2	-17.5	-14.6
WGCV-413 × PKV 081 Bt	37.46	32.54	35.0	33.47	-4.4	-10.7	-8.7	-5.5
WGCV-419 × WGCV Bt-108	30.78	31.54	31.2	35.66	14.4	13.1	-2.7	0.7
WGCV-419 × PKV 081 Bt	30.78	32.54	31.7	36.39	14.9	11.8	-0.8	2.7
WGCV Bt-60 × WGCV Bt-108	28.78	31.54	30.2	35.09	16.3	11.2	-4.3	-1.0
WGCV Bt-60 × PKV 081 Bt	28.78	32.54	30.7	31.92	4.1	-1.9	-13.0	-9.9
WGCV-372 × WGCV Bt-108	35.36	31.54	33.5	29.85	-10.8	-15.6	-18.6	-15.7
WGCV-372 × PKV 081 Bt	35.36	32.54	34.0	36.10	6.3	2.1	-1.5	1.9
Check-1 RCH 929	36.67							
Check-2 Swift	35.43							

**Table 10.** Comparison of heterotic effects of F<sub>1</sub> hybrids over mid-parent, better parent and standard checks (RCH 929 and Swift) for net plot yield/kg

F <sub>1</sub> hybrids	Line	Tester	Mid parent	F <sub>1</sub>	Percentage increase (+) or decrease (-) of F <sub>1</sub> over			
					Mid parent	Better parent	RCH 929	Swift
Co-17 × WGCV Bt-108	2.22	2.67	2.4	3.26	33.4	22.2	-17.4	1.6
Co-17 × PKV 081 Bt	2.22	2.60	2.4	2.26	-6.3	-13.1	-42.8	-29.6
WGCV-252 × WGCV Bt-108	2.49	2.67	2.6	3.22	24.7	20.5	-18.6	0.2
WGCV-252 × PKV 081 Bt	2.49	2.60	2.5	3.40	33.4	30.6	-14.0	5.8
WGCV-413 × WGCV Bt-108	2.80	2.67	2.7	2.65	-3.3	-5.5	-33.0	-17.6
WGCV-413 × PKV 081 Bt	2.80	2.60	2.7	2.53	-6.1	-9.5	-35.8	-21.0
WGCV-419 × WGCV Bt-108	2.35	2.67	2.5	2.81	11.9	5.1	-28.9	-12.5
WGCV-419 × PKV 081 Bt	2.35	2.60	2.5	2.59	4.5	-0.5	-34.5	-19.4
WGCV Bt-60 × WGCV Bt-108	1.51	2.67	2.1	3.23	54.4	20.9	-18.3	0.5
WGCV Bt-60 × PKV 081 Bt	1.51	2.60	2.1	3.34	62.4	28.3	-15.5	3.9
WGCV-372 × WGCV Bt-108	2.29	2.67	2.5	2.81	13.3	5.2	-28.9	-12.5
WGCV-372 × PKV 081 Bt	2.29	2.60	2.4	2.52	3.0	-3.1	-36.2	-21.6
Check-1 RCH 929	3.95							
Check-2 Swift	3.21							

plant height (4.442\*) and a small positive influence on lint index (0.008). Conversely, WGCV-413 was the weakest general combiner for yield with a significant negative GCA effect (-0.692\*\*\*) and also showed a negative influence on ginning outturn (-1.629\*). For the other traits, lines Co-17 and WGCV-252 generally had non-significant or negative GCA values, indicating lesser utility for yield improvement. WGCV Bt-108 was the least favourable tester for plant height (-4.442\*) and generally had weak or non-significant effects across traits.

Among the parents studied, WGCV-372 emerged as the best general combiner for yield, exhibiting highly significant positive GCA effects for net plot yield (0.718\*\*\*) and boll weight (0.529\*\*), indicating its strong additive gene contribution for yield improvement. WGCV Bt-60 also showed significant positive GCA effects for important yield components, including boll weight (0.122), lint index (0.767\*\*), seed index (1.429\*\*) and moderate gains in net plot yield (0.173), reflecting multi-trait superiority. Among testers, PKV 081 Bt displayed the strongest positive GCA for plant height (4.442\*) and a slight positive influence on lint index, making it useful

for improving plant stature and fiber quality traits. These results highlight the importance of parental GCA effects in selecting elite lines for hybrid breeding aimed at enhancing cotton yield and quality.

Among the hybrids, WGCV Bt-60 × WGCV Bt-108 showed strong positive SCA effects for plant height (7.342) and number of bolls per plant (1.305), indicating its potential to contribute favourable specific gene interactions for these traits. Conversely, its reciprocal cross WGCV Bt-60 × PKV 081 Bt exhibited significantly negative SCA effects for the same traits (-7.342 for plant height and -1.305 for bolls per plant), reflecting the importance of parent combination in hybrid performance. Similarly, WGCV-413 × PKV 081 Bt showed strong positive SCA for plant height (9.258), number of monopodia (0.092), sympodia (0.433) and bolls per plant (0.682), while its reciprocal cross showed negative effects, highlighting specific parent tester interactions impacting trait expression. WGCV-419 crosses also exhibited complementary opposite SCA effects in monopodia and sympodia. In terms of yield components, WGCV-252 × WGCV Bt-108 had significant positive SCA for lint index (0.633)

**Table 11.** General combining ability effects of parental lines and testers for yield and yield-related traits in cotton

Source	Plant height	Number of monopodia per plant	Number of sympodia per plant	Number of bolls per plant
<b>Lines</b>				
Co-17	-1.142	-0.858**	0.767	0.147
WGCV-252	-0.842	0.292	-0.983	0.322
WGCV-413	-4.992	1.242***	-0.433	-1.628*
WGCV-419	2.408	-0.408	0.317	-0.178
WGCV Bt-60	3.308	-0.608*	1.467*	0.285
WGCV-372	1.258	0.342	-1.133	1.052
<b>Testers</b>				
WGCV Bt-108	-4.442*	-0.058	-0.617	-0.285
PKV 081 Bt	4.442*	0.058	0.617	0.285

\**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001.

**Table 12.** General combining ability effects of parental lines and testers for yield and yield-related traits in cotton

Source	Boll weight (g)	Lint index (g)	Seed index (g)	Ginning outturn (%)	Net plot yield/ kg
<b>Lines</b>					
Co-17	-0.346	-0.108	-1.471**	3.171***	-0.234
WGCV-252	-0.198	-0.508*	-1.246**	0.396	-0.012
WGCV-413	-0.346	-0.333	-0.021	-1.629*	-0.692***
WGCV-419	0.239	0.092	0.929*	-1.654*	0.046
WGCV Bt-60	0.122	0.767**	1.429**	0.146	0.173
WGCV-372	0.529**	0.092	0.379	-0.429	0.718***
<b>Testers</b>					
WGCV Bt-108	0.183	-0.008	0.196	-0.388	0.025
PKV 081 Bt	-0.183	0.008	-0.196	0.388	-0.025

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

**Table 13.** Specific combining ability effects of  $F_1$  hybrid for yield and yield-related traits in cotton

Cross	Plant height	Number of monopodia per plant	Number of sympodia per plant	Number of bolls per plant
Co-17 × WGCV Bt-108	2.092	-0.092	-1.133	-1.227
Co-17 × PKV 081 Bt	-2.092	0.092	1.133	1.227
WGCV-252 × WGCV Bt-108	1.492	0.158	0.317	0.728
WGCV-252 × PKV 081 Bt	-1.492	-0.158	-0.317	-0.728
WGCV-413 × WGCV Bt-108	-9.258	-0.092	-0.433	-0.682
WGCV-413 × PKV 081 Bt	9.258	0.092	0.433	0.682
WGCV-419 × WGCV Bt-108	-2.458	0.558	0.717	-0.692
WGCV-419 × PKV 081 Bt	2.458	-0.558	-0.717	0.692
WGCV Bt-60 × WGCV Bt-108	7.342	-0.142	-0.333	1.305
WGCV Bt-60 × PKV 081 Bt	-7.342	0.142	0.333	-1.305
WGCV-372 × WGCV Bt-108	0.792	-0.392	0.867	0.568
WGCV-372 × PKV 081 Bt	-0.792	0.392	-0.867	-0.568

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

**Table 14.** Specific combining ability effects of  $F_1$  hybrid for yield and yield-related traits in cotton

Cross	Boll weight (g)	Lint index (g)	Seed index (g)	Ginning outturn (%)	Net plot yield/ kg
Co-17 × WGCV Bt-108	0.338	-0.067	-0.096	-0.288	-0.158
Co-17 × PKV 081 Bt	-0.338	0.067	0.096	0.288	0.158
WGCV-252 × WGCV Bt-108	-0.045	0.633	-0.271	3.737**	0.200
WGCV-252 × PKV 081 Bt	0.045	-0.633	0.271	-3.737**	-0.200
WGCV-413 × WGCV Bt-108	-0.368	-0.592	0.004	-2.888*	-0.415
WGCV-413 × PKV 081 Bt	0.368	0.592	-0.004	2.888*	0.415
WGCV-419 × WGCV Bt-108	0.448	0.483	1.054	-0.112	0.013
WGCV-419 × PKV 081 Bt	-0.448	-0.483	-1.054	0.112	-0.013
WGCV Bt-60 × WGCV Bt-108	0.070	-0.442	0.004	-1.813	0.475*
WGCV Bt-60 × PKV 081 Bt	-0.07	0.442	-0.004	1.813	-0.475*
WGCV-372 × WGCV Bt-108	-0.443	-0.017	-0.696	1.363	-0.115
WGCV-372 × PKV 081 Bt	0.443	0.017	0.696	-1.363	0.115

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

and ginning outturn (3.737\*\*), while its reciprocal showed negative effects, accentuating the specificity of combining ability. Notably, WGCV Bt-60 × WGCV Bt-108 manifested a significant positive SCA for net plot yield/kg (0.475\*), indicating its potential as a high-yielding hybrid.

The hybrids WGCV Bt-60 × WGCV Bt-108, WGCV-413 × PKV 081 Bt and WGCV-252 × WGCV Bt-108 demonstrated strong SCA effects for important agronomic and fiber traits. WGCV Bt-60 × WGCV Bt-108 showed significant positive SCA for plant height (7.342), number of bolls per plant (1.305) and net plot yield (0.475\*). WGCV-413 × PKV 081 Bt exhibited high positive SCA effects for plant height (9.258), monopodia (0.092), sympodia (0.433) and bolls per plant (0.682), highlighting its utility in improving plant architecture and yield. Meanwhile, WGCV-252 × WGCV Bt-108 recorded significant SCA for lint index (0.633) and ginning outturn (3.737\*\*). These crosses underscore the importance of specific parent combinations for maximizing hybrid performance in cotton breeding programs.

Significant negative effects on plant height showing in the GCA of WGCV Bt-108 and in certain hybrid combinations such as WGCV-413 × WGCV Bt-108 (heterosis) and WGCV Bt-60 × PKV 081 Bt (SCA), which could be valuable for breeding compact genotypes.

## Discussion

Cotton (*Gossypium* spp.) holds a paramount position as a global agricultural commodity, serving as the primary natural fiber source and a significant oilseed crop (20). The escalating demand from the textile industry, coupled with challenges posed by climate change and resource scarcity, necessitates continuous genetic improvement in cotton cultivars. Understanding the relative importance of GCA and SCA is critical for developing effective breeding strategies. If additive gene action (GCA) is predominant for a trait, breeders can focus on improving parental lines through recurrent selection. If non-additive gene action (SCA) is more significant, hybrid breeding programs that exploit specific cross combinations are more appropriate (21). Both additive (GCA) and non-additive (SCA) gene actions influence cotton traits, with their relative importance varying by trait. This highlights the need for sophisticated, tailored breeding strategies (22).

The present study identifies WGCV-413 as a promising parent for breeding short plant types, as indicated by its strong negative GCA and the superior negative heterosis of its crosses, particularly WGCV-413 × WGCV Bt-108. The significant SCA effects observed for plant height reduction confirm that non-additive gene

effects, likely due to dominance or over-dominance, play a crucial role in controlling plant stature. This finding aligns with previous research, which reported the predominance of non-additive gene action for plant height and emphasized the importance of short plant architecture for modern, high-density planting systems (23). Similarly, reported that non-additive gene effects were more prominent than additive effects for plant height, suggesting that hybrid breeding is an effective strategy for managing this trait (24). The strong negative heterosis observed for plant height reduction is a highly desirable outcome, as reduced plant stature enhances lodging resistance and facilitates easier management and mechanized harvesting, a critical aspect of modern cotton production (25).

The genotypic analysis indicates that the number of monopodial branches is primarily controlled by both additive and non-additive gene effects. Among the genotypes evaluated, Co-17 demonstrated the highest GCA for reduced monopodial branching. This is particularly valuable for developing a compact plant type with fewer vegetative branches and more reproductive (sympodial) branches, leading to a more efficient canopy. The significant SCA of the WGCV-372 × WGCV Bt-108 cross for monopodial suppression further supports the role of non-additive gene action in this trait. This is consistent with the findings of previous research, which also reported that non-additive gene action was predominant for the number of monopodia (26). In contrast, previous studies highlighted the importance of non-additive and additive gene action for monopodial branches (27, 28). The use of a parent like Co-17 with a low number of monopodia is an effective breeding strategy to develop varieties with an upright growth habit, better light interception and improved yield potential.

The superior positive heterosis was observed for boll number in crosses involving WGCV-372 as the female parent. The outstanding performance of WGCV Bt-60 × WGCV Bt-108 indicates a superior specific combining ability, suggesting that hybrid breeding is the most effective approach for enhancing this trait. This finding is corroborated by numerous studies that have identified boll number as a major component of yield, predominantly influenced by non-additive gene action (29, 30). Another study also reported that boll number was significantly influenced by non-additive gene action, emphasizing the importance of selecting crosses with high SCA effects for developing high-yielding hybrids (31). This trait, being a direct contributor to yield, is a primary target in most cotton breeding programs and the identified cross provides a strong foundation for future yield enhancement.

The results demonstrate that WGCV-372 is a strong general combiner for boll weight, while the cross WGCV-419 × WGCV Bt-108 exhibits superior specific combining ability. This indicates that both additive and non-additive gene actions control boll weight, which is a key component of seed cotton yield. The findings of the study align with previous studies that reported the significant role of non-additive gene action for boll weight in various crosses of upland cotton (32). It was also found that boll mass was largely influenced by dominant gene effects, confirming that heterosis breeding is a viable strategy for improving this trait (33). While hybrids of the study showed promising performance, the negative heterosis against the commercial variety 'Swift' suggests that some existing cultivars possess superior genetic potential for boll weight, providing a benchmark for future breeding efforts (29). Thus, the identified parents and crosses can be utilized to improve boll weight in

breeding programs targeting increased yield.

The analysis revealed that WGCV Bt-60 is a strong general combiner for lint index, while the cross WGCV-252 × WGCV Bt-108 had superior SCA. This indicates that both additive and non-additive gene effects influence this crucial fiber quality trait. The results are consistent with the findings of previous research, which reported significant GCA and SCA effects for lint percentage, underscoring the potential for improving this trait through both parental selection and hybrid development (23). Furthermore, previous studies have highlighted the importance of non-additive gene action for lint index, suggesting that heterosis breeding can be effectively employed to enhance lint production characteristics (13, 29). The identification of WGCV Bt-60 as a superior parent for GCA suggests that this line can be used in crosses to accumulate introgressed genes for lint index, leading to the development of superior breeding populations.

This study identified WGCV Bt-60 as having the strongest GCA for seed index, while the cross WGCV-419 × WGCV Bt-108 showed superior SCA. These results indicate that both additive and non-additive gene effects are significant for seed index in cotton. This is supported by several recent studies, including one that reported significant heterosis and the involvement of non-additive gene action for 100-seed weight (24). Similarly, previous research noted the importance of both additive and non-additive gene effects for seed index, with specific crosses showing high SCA effects (34). The substantial GCA of WGCV Bt-60 suggests its utility as a valuable parent for developing varieties with heavier seeds, which can contribute to both better germination and higher oil content (35).

The line Co-17 possesses the strongest GCA for GOT, while the cross WGCV-252 × WGCV Bt-108 exhibits superior SCA. These results demonstrate that both additive and non-additive gene effects govern fiber recovery in cotton. The presence of significant non-additive gene effects is further supported by the positive heterosis observed in several crosses. These findings are in line with previous reports, which indicated that non-additive gene action is prominent for GOT and that both GCA and SCA play significant roles in improving this trait (24). The strong GCA of Co-17 makes it a suitable parent for improving ginning outturn through selection, while the high SCA of the WGCV-252 × WGCV Bt-108 cross presents a valuable hybrid combination for direct commercial exploitation (13).

The line WGCV-372 is a promising parent for yield improvement, as evidenced by the maximum positive GCA effect. The outstanding performance of WGCV Bt-60 × WGCV Bt-108 with its superior SCA effects confirms the critical role of non-additive gene action in controlling yield potential. Many studies report that heterosis and SCA are primary drivers of yield enhancement (23, 29). The high heterotic values observed in this study are comparable to the findings of other researchers who have successfully identified superior hybrids with significant yield gains over parental lines (13, 35). The identification of WGCV-372 as a strong general combiner for yield further suggests its utility in developing improved breeding populations through selection, thereby offering a dual approach for both immediate hybrid deployment and long-term varietal development.

## Conclusion

The line × tester analysis revealed significant genetic variability among genotypes for most yield and fiber traits, with crosses and line × tester interactions showing strong significance.



Among the studied hybrids, WGCV Bt-60 × PKV 081 Bt exhibited highest positive heterosis for yield, along with maximum heterosis in the number of bolls per plant. WGCV-252 × PKV 081 Bt recorded the highest heterosis for boll weight, while WGCV-Bt-60 × WGCV Bt-108 showed notable improvement in ginning outturn. The results demonstrate that both additive and non-additive gene effects contribute significantly to yield and quality traits in American cotton. Hybrids such as WGCV Bt-60 × PKV 081 Bt and WGCV-252 × PKV 081 Bt warrant further evaluation across environments to assess stability and adaptability. For long-term population improvement, the superior GCA parents (WGCV-372 and Co-17) should be strategically deployed in recurrent selection or diallel selective mating programs to accumulate desirable additive effects. Furthermore, parents exhibiting negative GCA for plant height, such as WGCV-413 and WGCV Bt-108 are valuable resources for breeding short-statured, lodging-tolerant varieties. Future research should focus on multi-location trials, advanced generation studies and integration of molecular markers to accelerate the development of high-yielding, quality-oriented cotton hybrids.

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## Authors' contributions

YP executed the research work, designed methodology and drafted the initial manuscript. YP supervised the whole research work. YP & BE analysed the data, prepared figures and tables, results interpreted and drafted manuscript. YP, BE, GV, SO, DA, RUR participated and monitored in the field trials and reviewed the manuscript. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interests to declare.

**Ethical issues:** None

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