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Combining ability and heterosis in bell pepper grown in the north-western hills of India

Divya Arti*, D.K. Mehta, Amit Vikram, Ramesh Kumar and Omkar Aralikatti

Department of Vegetable Science, Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan-173 230, Himachal Pradesh, India.

ABSTRACT

In this study, 51 hybrids were developed using Line × Tester mating design, including 17 lines and 3 testers. Analysis of variance for combining ability indicated that differences among parents were significant for all the characters under study except for days to first picking. The σ^2 SCA were higher in magnitude than σ^2 GCA for almost all the traits except for days to first picking, which indicates the predominant role of non-additive gene action. Lines *viz*., UHF CAP-23, UHF CAP-1 and UHF CAP-22 and 2 testers *viz*., Yolo Wonder and California Wonder were the most promising for yield per plant and the majority of yield contributing traits. The SCA effects for fruit yield per plant were significantly desirable in UHF CAP-30 × California Wonder, UHF CAP-2 × Solan Bharpur and UHF CAP-29 × Solan Bharpur. Heterosis over the standard check and other genetic parameters 3 cross combinations *viz*., UHF CAP-30 × California Wonder, UHF CAP-23 × Yolo Wonder and UHF CAP-22 × California Wonder performed well for a majority of yield and yield contributing traits. These hybrids can be recommended for cultivation after multi-locational testing.

Key words : *Capsicum annuum* var. *grossum*, Gene action, GCA, SCA, Heterosis

INTRODUCTION

Bell pepper (*Capsicum annuum* var. *grossum*) is a popular vegetable known for its pleasant flavour and sweet aroma. It belongs to the *Solanaceae* family (2n=24) and originated in South and Central America. Green bell pepper is an excellent source of ascorbic acid and a fair source of carotenoids (Haytowitz and Matthews, 8). In addition, peppers are also rich in flavonoids (Lee *et al*., 10) and other phytochemicals. Sweet pepper fruits are generally blocky, square, thick-fleshed, 3-4 lobed and non-pungent. Perfect fruit shape, size, colour, and mild taste are the main quality parameters that make developing new genotypes/ varieties/hybrids very challenging. Emphasis is being given to developing hybrids worldwide to meet the ever-growing demand for sweet peppers. Bell pepper is a leading off-season vegetable crop highly suitable for protected cultivation which generates cash revenues for the farmers by selling high-quality produce in the neighbouring states and metropolitan cities. Hence, it is essential to develop hybrids because they are high-yielding and uniform in shape and size. Therefore, there is an urgent need to strengthen the crop improvement program for developing new varieties or hybrids capable of satisfying the needs of farmers and consumers. Heterosis and combining ability are two important considerations in hybrid development. Combining ability refers to the ability of lines or parents to combine well during the hybridization process so that desirable genes or characters get transmitted to their progenies (Fasahat *et al*., 7). The magnitude and direction of heterosis are crucial factors for crop improvement depending on the objectives of the hybrid breeding program. The objective of the present study was to assess the combining ability and to estimate heterosis in bell pepper to select the economically desirable hybrids.

MATERIALS AND METHODS

The experiment was conducted at the Research Farm Department of Vegetable Science, Dr. YS Parmar University of Horticulture and Forestry, Nauni, Solan (Himachal Pradesh), India at 35.5° North latitude and 77.8° East longitude at an altitude of 1,270 meters above mean sea level. The area corresponds to the mid-hill zone of Himachal Pradesh, having a sub-humid, sub-temperate with cool winters. Mean temperature during the cropping season varied from 20.1 °C to 24.9 °C while the relative humidity ranged between 55.0 per cent to 85.0 per cent and received rainfall of 1100-1300 mm annually, most of which occurs during the South-West monsoon (June- August). The experimental material comprised 17 lines and 3 testers of bell pepper as parents. Crosses were made as per the Line × Tester mating design and evaluated in a randomized complete block design with three replications during the year

*Corresponding author: divyaarti091993@gmail.com

2021. The saplings were space planted at 60 cm inter-row and 45 cm intra-row. The standard cultural practices were followed to raise the healthy crop stand as recommended in the package of practices of Vegetable Crops (Anonymous, 2).

Data were recorded for the different traits, namely, days to 50 per cent flowering (DF), days to first picking (DP), plant height (PH), harvest duration (HD), plant spread (PS), number of fruits per plant (NFP), fruit length (FL), fruit breadth (FB), fruit weight (FW), fruit yield per plant (FYP), number of lobes per fruit (NBF), pericarp thickness (PT), number of seeds per fruit (NSF) and ascorbic acid (AA). The observations were recorded on ten randomly selected plants for the parents and hybrids. The data were statistically analyzed as per the procedure given by Panse and Sukhatme (13). Combining ability analysis for the Line × Tester mating design was done using Kempthorne's (9) statistical methods I and II. The heterosis estimates given by Allard (1) were calculated as the deviation of the F_1 means from the better parent, mid-parent, and standard check.

Heterosis over better parent (BP) = $[(\overline{F}_1 - \overline{BP}) / \overline{BP}] \times 100$

Heterosis over the standard check (SC) = $[(\overline{F}_1 - \overline{SC}) / \overline{SC}] \times 100$

The test of significance for heterosis was done through a t-test at an error degree of freedom. Data analysis for combining ability and heterosis was performed using the computer software program Windowstat 9.2 (INDOSTAT Services, Hyderabad, India) and MS Excel.

RESULTS AND DISCUSSION

In the utilization of hybrid breeding, the evaluation of combiners is a major step for the selection of crossing parents affecting the performance of the hybrid. Boyaci *et al*. (3) also reported the importance of parents' general combining ability (GCA) and obtained cross combinations with higher yields than commercial cultivars**.** General combining ability is linked to the additive effects, whereas specific combining ability (SCA) is linked to the dominance and epistatic effects (Sprague and Tatum, 15). Understanding the genetic potential of a population and deciding on the breeding strategy to be used in a given population requires knowledge of the nature and magnitude of gene action. In addition, gene action is valuable in establishing the commercial viability of heterosis and pure line isolation.

Analysis of variance for combining ability (Table 1) indicated that parents showed significant differences for all the characters under study. The variances due to GCA were significant for all the characters. In contrast, testers also exhibited significant differences for characters except for days to first picking, thereby

Table 1. Analysis of variance for combining ability for yield and yield contributing traits in bell pepper.

Source	Df	DF	DP	PH	HD	PS	NFP	FL	FB	FW	FLIP	NLPF	PT	NSPF	AA
Replications	2	13.72*	64.52*	0.17	20.53*	17.04*	215.158	0.05*	0.03	12.01*	7232.41*	0.03	0.00*	2941.20*	45.03*
Crosses	50	13.08*	12.11*	117.93*	11.18*	41.52*	36.75*	2.07*	1.67*	703.38*	283491.33*	0.40*	0.01*	28135.84*	393.79*
Lines	16	21.96*	21.39*	195.41*	15.42*	74.84*	80.30*	2.75*	2.66*	466.69*	308167.91*	0.71*	0.01*	16845.98*	395.15*
Tester	2	24.16*	20.12	128.49*	23.53*	20.83*	91.16*	12.09*	7.31*	7836.15*	1474118.42*	0.30*	0.01*	213555.80*	4599.54*
Line×Tester	32	7.95*	6.97	78.54*	8.29*	26.15*	11.58*	1.10*	0.83*	375.93*	196738.84*	0.26*	0.01*	22192.02*	130.26*
Error	100	0.78	7.08	0.40	4.14	1.37	0.43	0.02	0.02	2.88	847.61	0.03	0.00	84.24	0.33

*Significant at a 5 % level of significance

DF, days to 50 percent flowering; DP, days to first picking; PH, plant height (cm); HD, harvest duration; PS, plant spread (cm); NFP, number of fruits per plant; FL, fruit length (cm); FB, fruit breadth (cm); FW, fruit weight (g); FYP, fruit yield per plant (g); NLPF, number of lobes per fruit; PT, pericarp thickness (mm); NSPF, number of seeds per fruit; AA, Ascorbic acid (mg/100gm) indicating a good amount of genetic variability among the parents for all the characters studied. Further, the interaction between line × tester (SCA) was significant for most characters except for days to first picking. This indicated the predominant role of both additive and non-additive gene effects in the inheritance of these characters.

Based on estimates of GCA effects, the lines UHF CAP-23, UHF CAP-1, and UHF CAP-22 and two testers *viz*., Yolo Wonder and California Wonder, were the most promising for fruit yield per plant and the majority of its component traits like earliness, harvest duration, number of fruits per plant, fruit length, fruit breadth and fruit weight. Therefore, it becomes important to consider the GCA effects for choosing the parents for crossing. SCA effects are useful for identifying specific crosses with desirable traits. Among the 51 cross combinations, the hybrid UHF CAP-26 × California Wonder (-3.34) showed the highest negative SCA effect for days to 50 per cent flowering whereas, UHF CAP-31 × Solan Bharpur (-3.37) had a maximum negative SCA effect for days to first picking. Crosses with significant negative SCA effects are considered good specific combiners for days to 50 per cent flowering and days to first picking, which is desirable for early harvest. UHF CAP-33 × Yolo Wonder possessed the highest SCA effect for characters like plant height, plant spread, number of lobes per fruit and ascorbic acid. None of the hybrids possessed a significant SCA effect for harvest duration. An appraisal of SCA effects for the number of fruits per plant and fruit yield per plant in F_1 indicated that UHF CAP-30 × California Wonder was the best specific combiner. UHF CAP-3 × California Wonder (1.21) exhibited the desirable specific combiner for fruit length. The hybrid UHF CAP-24 × Solan Bharpur (0.70) possessed the highest SCA effect for character fruit breadth. UHF CAP-25 × Yolo Wonder exhibited the best specific combiner for fruit weight, whereas UHF CAP-3 × Solan Bharpur (0.09) was the best for pericarp thickness. For the number of seeds per fruit, UHF CAP-3 × Yolo Wonder (202.47) possessed the highest SCA effect for this character.

Estimates of GCA, SCA variances, additive (σ^2A) and dominant ($\boldsymbol{\sigma}^2$D) variance derived by the line × tester analysis were analyzed (Table 2) to comprehend the nature of gene action. SCA variances were higher in magnitude as compared to the GCA variances (average) for most of the traits, *viz*., days to 50 per cent flowering, plant height, harvest duration, plant spread, number of fruits per plant, fruit length, fruit breadth, fruit weight, fruit yield

Table 2. Estimates of genetic components of variance for different horticultural and quality traits in bell pepper.

Character (s)	σ^2 GCA (Lines)	Σ^2 GCA (Testers)	σ^2 GCA (Average)	σ^2 SCA	σ^2 A	σ^2 D	σ^2 A/ σ^2D	Degree of dominance	Heritability (Narrow Sense) %	Predictability ratio
DF	1.56	0.32	0.06	2.39	0.12	2.39	0.05	3.65	4.28	-0.18
DP	1.60	0.26	0.06	-0.04	0.12	-0.04	-3.12	9.67	4.92	-0.02
PH	12.99	0.98	0.44	26.04	0.89	26.04	0.03	7.30	3.29	0.04
HD	0.79	0.30	0.03	1.38	0.07	1.38	0.05	13.99	2.28	-0.01
PS	5.41	-0.10	0.17	8.26	0.35	8.26	0.04	6.41	3.83	0.05
NFP	7.64	1.56	0.28	3.72	0.57	3.72	0.15	3.54	12.85	0.14
FL	0.18	0.22	0.01	0.36	0.02	0.36	0.06	5.21	5.64	0.07
FB	0.20	0.13	0.01	0.27	0.02	0.27	0.07	4.92	6.49	0.08
FW	10.08	146.28	3.70	124.35	7.40	124.35	0.06	5.61	5.57	0.06
FYP	12381.00	25046.66	979.59	65297.08	1959.18	65297.08	0.03	8.00	2.30	0.03
NLPF	0.05	0.00	0.00	0.08	0.00	0.08	0.04	6.45	3.75	0.05
PT	0.06	0.01	0.00	0.36	0.00	0.26	0.00	11.97	1.28	0.01
NSPF	-594.00	3752.23	67.12	7369.26	134.23	7369.26	0.02	9.14	2.30	0.02
AA	29.43	87.63	2.98	43.31	5.95	43.31	0.14	1.92	12.06	0.35

DF, days to 50 percent flowering; DP, days to first picking; PH, plant height (cm); HD, harvest duration; PS, plant spread (cm); NFP, number of fruits per plant; FL, fruit length (cm); FB, fruit breadth (cm); FW, fruit weight (g); FYP, fruit yield per plant (g); NLPF, number of lobes per fruit; PT, pericarp thickness (mm); NSPF, number of seeds per fruit; AA, Ascorbic acid (mg/100gm) per plant, number of lobes per fruit, number of seeds per fruit and ascorbic acid signifying the prevalence of non-additive gene action influencing the attributes. The dominant variance (σ^2D) magnitude was higher than the corresponding additive variance (σ^2A) for most characters, showing non-additive gene action except for days to first picking. Other authors have also recorded non-additive gene action for fruit yield and yield-contributing traits (Sood and Kumar, 14; Pandey *et al.*, 12; Dhillon *et al.*, 5). The preponderance of non-additive gene action in the inheritance of all the traits studied suggested the exploitation of heterosis breeding to improve these traits and sufficient hybrid vigour in different cross combinations.

Heterosis breeding is one of bell pepper's most significant tools to exploit genetic diversity. The nature and magnitude of heterobeltiosis help identify promising cross combinations and their exploitation to obtain better transgressive segregants (Chandel *et al.*, 4). A wide range of heterosis over better parent and the standard check was observed in the F_1 generation (Table 3). Out of 51 hybrids, 13 cross combinations showed significantly negative heterosis over a better parent, whereas, in economic heterosis, 16 cross combinations recorded desirable significant negative heterosis for days to 50 per cent flowering. Early maturing strains are of immense value in capturing early markets. None of the 51 cross combinations had significant negative heterosis over the better parent. Only UHF CAP-1 × Yolo Wonder (-5.78 %) significantly showed the highest negative heterosis in economic heterosis. Out of 51 hybrids, 8 had significant positive heterosis over the better parent, whereas 17 had significantly positive economic heterosis for plant height. Among 51 hybrids, 5 and 11 crosses showed significant positive better parent and standard heterosis for plant height, respectively. For plant spread, out of 51 cross combinations, 14 crosses had significant positive heterobeltiosis, whereas, in standard heterosis, only 2 crosses exhibited significant positive heterosis. Twenty-seven hybrids have shown significant positive better parent heterosis, whereas 15 hybrids showed significant positive economic heterosis for the number of fruits per plant.

In heterobeltiosis, 4 crosses reported significant positive heterosis for fruit length, whereas 37 hybrids had significant positive heterosis over the standard check. For fruit breadth, 8 and 14 crosses have exhibited significant positive heterosis over better parent and standard check, respectively. For fruit weight, 11 hybrids were reported with significant positive heterosis over the better parent, and for the check, 7 crosses had significant positive values.

Table 3. Top three parents and cross combinations based on their per se performance and heterotic values.

Characters	*Per se* performance		Heterosis	
	Parents	Crosses	BP	SC
DF	UHF CAP-1 (-2.98)	UHF CAP-26 × California Wonder (-3.34)	UHF CAP-26 × California Wonder (-19.62 %)	UHF CAP-26 × California Wonder (-8.63 %)
	UHF CAP-4 (-2.86)	UHF CAP-22 × California Wonder (-2.58)	UHF CAP-22 × California Wonder (-10.42 %)	UHF CAP-4 × Solan Bharpur (-7.19 %)
	UHF CAP-3 (-1.66)	UHF CAP-3 × Yolo Wonder (-1.75)	UHF CAP-4 × Solan Bharpur (-7.19 %)	UHF CAP-22 × California Wonder (-7.19 %)
DP	UHF CAP-1 (-4.71)	UHF CAP-31 × Solan Bharpur (-3.37)	-	UHF CAP-1 × Yolo Wonder (-5.78 %)
	UHF CAP-20 (-2.26)	UHF CAP-13 × Yolo Wonder (-3.25)		
PH	UHF CAP-4 (8.43)	UHF CAP-33 × Yolo Wonder (12.74)	UHF CAP-29 × Yolo Wonder (21.10 %)	UHF CAP-33 × Yolo Wonder (43.21 %)
	UHF CAP-33 (6.74)	UHF CAP-22 × Solan Bharpur (7.63)	UHF CAP-2 × Yolo Wonder (14.73 %)	UHF CAP-3 × Yolo Wonder (23.87 %)
	UHF CAP-3 (5.54)	UHF CAP-23 × Yolo Wonder (5.85)	UHF CAP-4 × Yolo Wonder (13.82 %)	UHF CAP-4 × Yolo Wonder (22.02 %)
HD	UHF CAP-22 (1.99)	-	UHF CAP-2 × California Wonder (13.50 %)	UHF CAP-22 × Yolo Wonder (7.04 %)
	Yolo Wonder (0.78)		UHF CAP-1 × California Wonder (12.49 %)	UHF CAP-2 × Yolo Wonder (6.16 %)
			UHF CAP-33 × California Wonder (7.84 %)	UHF CAP-33 × Yolo Wonder (6.16 %)

Contd...

Table 3 contd...

Characters	*Per se* performance		Heterosis	
	Parents	Crosses	BP	SC
PS	UHF CAP-25 (6.96)	UHF CAP-33 × Yolo Wonder (6.43)	UHF CAP-25 × Yolo Wonder (33.69 %)	UHF CAP-33 × Yolo Wonder (9.24 %)
	UHF CAP-33 (4.19)	UHF CAP-13× California Wonder (4.42)	UHF CAP-33 × Yolo Wonder (31.19 %)	UHF CAP-25 × Yolo Wonder (5.91 %)
	UHF CAP-13 (2.73)	UHF CAP-22 × Yolo Wonder (4.24)	UHF CAP-25 × Solan Bharpur (15.57 %)	
NFP	UHF CAP-23 (7.89)	UHF CAP-30 × California Wonder (4.51)	UHF CAP-23 × California Wonder (95.12 %)	UHF CAP-23 × Solan Bharpur (104.92 %)
	UHF CAP-22 (2.99)	UHF CAP-31 × Solan Bharpur (3.01)	UHF CAP-23 × Yolo Wonder (86.18 %)	UHF CAP-23 × California Wonder (96.72 %)
	UHF CAP-24 (2.42)	UHF CAP-25 × Solan Bharpur (2.61)	UHF CAP-32 × California Wonder (61.61 %)	UHF CAP-23 × Yolo Wonder (87.70 %)
FL	UHF CAP-2 (0.84)	UHF CAP-3 × California Wonder (1.21)	UHF CAP-21 × Yolo Wonder (6.49 %)	UHF CAP-3 × California Wonder (40.68 %)
	UHF CAP-1 (0.82)	UHF CAP-31 × Solan Bharpur (0.83)	UHF CAP-3 × California Wonder (6.41 %)	UHF CAP-13 × Yolo Wonder (40.68 %)
	UHF CAP-21(0.67)	UHF CAP-13 × Yolo Wonder (0.75)	UHF CAP-21 × Solan Bharpur (5.17 %)	UHF CAP-21 × Yolo Wonder (38.98 %)
FB	UHF CAP-1(1.47)	UHF CAP-24 × Solan Bharpur (0.70)	UHF CAP-24 × Solan Bharpur (14.81 %)	UHF CAP-1 × Yolo Wonder (35.94 %)
	UHF CAP-2 (0.83)	UHF CAP-2 × Yolo Wonder (0.69)	UHF CAP-2 × Yolo Wonder (12.50 %)	UHF CAP-2 × Yolo Wonder (26.61 %)
	UHF CAP-4 (0.64)	UHF CAP-1 × Yolo Wonder (0.64)	UHF CAP-1 × Yolo Wonder (10.30 %)	UHF CAP-1 × California Wonder (19.45 %)
FW	UHF CAP-2 (16.79)	UHF CAP-25 × Yolo Wonder (16.72)	UHF CAP-2 × Solan Bharpur (32.62 %)	UHF CAP-25 × Yolo Wonder (12.32 %)
	UHF CAP-1 (11.38)	UHF CAP-20 × Yolo Wonder (16.42)	UHF CAP-25 × Yolo Wonder (14.65 %)	UHF CAP-2 × California Wonder (5.89 %)
	UHF CAP-3 (4.84)	UHF CAP-30 × Solan Bharpur (13.56)	UHF CAP-31 × Solan Bharpur (12.53 %)	UHF CAP-2 × Yolo Wonder (5.36 %)
FYP	UHF CAP-23 (403.00)	UHF CAP-30 × California Wonder (587.24)	UHF CAP-31 × Solan Bharpur (120.98 %)	UHF CAP-30 × California Wonder (75.65 %)
	UHF CAP-1 (213.10)	UHF CAP-2 × Solan Bharpur (411.36)	UHF CAP-29 × Solan Bharpur (96.28 %)	UHF CAP-23 × Yolo Wonder (61.00 %)
	UHF CAP-22 (201.93)	UHF CAP-29 × Solan Bharpur (402.23)	UHF CAP-2 × Solan Bharpur (60.07 %)	UHF CAP-22 × California Wonder (57.58 %)
NLPF	UHF CAP-1 (0.26)	UHF CAP-33 × Yolo Wonder (0.68)	UHF CAP-31 × Solan Bharpur (80.00 %)	UHF CAP-1 × Yolo Wonder (17.14 %)
	UHF CAP-3 (0.19)	UHF CAP-21 × Yolo Wonder (0.49)	UHF CAP-29 × Solan Bharpur (11.76 %)	UHF CAP-3 × Solan Bharpur (14.29 %)
	UHF CAP-4 (0.17)	UHF CAP-24 × Solan Bharpur (0.33)	UHF CAP-4 × Solan Bharpur (8.33 %)	UHF CAP-4 × Solan Bharpur (11.43 %)
PT	UHF CAP-31 (0.06)	UHF CAP-3 × Solan Bharpur (0.09)	UHF CAP-33 × Yolo Wonder (56.78 %)	UHF CAP-33 × Yolo Wonder (23.33 %)
	UHF CAP-32 (0.05)	UHF CAP-26 × California Wonder (0.08)	UHF CAP-29 × Solan Bharpur (49.21 %)	UHF CAP-29 × Solan Bharpur (17.37 %)
	UHF CAP-2 (0.04)	UHF CAP-33 × Yolo Wonder (0.07)	UHF CAP-31 × Solan Bharpur (48.26 %)	UHF CAP-31 × Solan Bharpur (16.63 %)
NSPF	UHF CAP-24 (70.89)	UHF CAP-3 × Yolo Wonder (202.47)	UHF CAP-24 × California Wonder (210.54 %)	UHF CAP-3 × Yolo Wonder (51.06 %)
	UHF CAP-29 (69.59)	UHF CAP-29 × California Wonder (171.70)	UHF CAP-33 × California Wonder (107.61 %)	UHF CAP-22 × Solan Bharpur (50.31 %)
	UHF CAP-31 (34.79)	UHF CAP-20 × Yolo Wonder (87.94)	UHF CAP-29 × California Wonder (97.10 %)	UHF CAP-1× Solan Bharpur (50.24 %)
AA	UHF CAP-26 (10.29)	UHF CAP-33 × Yolo Wonder (16.96)	UHF CAP-30 × Solan Bharpur (8.46 %)	UHF CAP-32 × California Wonder (26.21 %)
	UHF CAP-32 (9.79)	UHF CAP-4 × Solan Bharpur (9.12)	UHF CAP-22 × California Wonder (8.14 %)	UHF CAP-26 × California Wonder (26.21 %)
	UHF CAP-23 (6.81)	UHF CAP-29 × California Wonder (7.61)	UHF CAP-1 × California Wonder (7.34 %)	UHF CAP-22 × California Wonder (25.48 %)

*Significant at a 5 % level of significance

Among 51 cross combinations, 26 F_1 hybrids had significant positive heterosis over the better parent, whereas, in standard heterosis, 28 hybrids were found with significant positive heterosis.

Among 51 hybrids, 4 hybrids resulted in significant positive heterosis over better parent and for the check, 11 crosses were found with significant positive heterosis for the number of lobes per fruit. For the number of seeds per fruit, significant positive heterosis was observed for 16 hybrids in better parent and 24 crosses in check, respectively. Twenty-three hybrids showed significant positive better parent heterosis, whereas 46 hybrids showed significant positive economic heterosis for ascorbic acid. As a result, elite inbreds with improved combining ability and heterosis could be exploited for efficient hybrid breeding. Similar findings of heterosis were also reported for fruit yield and other yield-contributing traits (Dhillon *et al*., 6; Nalwa and Kumar, 11; Varsha *et al*., 16). Therefore, estimating heterosis for quantitative traits could help to determine the most heterotic combinations. In bell pepper, to exploit the heterosis, it has been found that the selection of diverse parents may be valuable for obtaining heterotic hybrids in terms of horticultural traits also emphasized the selection of diverse parents resulting in superior outcomes owing to specific heterotic groups.

AUTHORS' CONTRIBUTION

Conceptualization and designing of the research work (DA, DKM, AV, RK); Execution of field/lab experiments and data collection (DA); Analysis of data and interpretation (DA, DKM, AV, RK); Preparation of manuscript (DA, DKM, AV, RK, OA).

DECLARATION

The authors declare that they have no potential conflict of interest.

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