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Original Research

Diallel Analysis and Selection of Hybrids for Nutritional Phytochemicals in *Capsicum Annuum* L.

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Abstract

Chili (*Capsicum annuum* L.) is an important functional food due to its main bioactive compound, capsaicin, and other nutritional phytochemicals. However, very few studies have been conducted to develop hybrids with a high content of nutritional phytochemicals. The present study involving six parents was conducted to identify superior hybrids with higher nutritional quality based on combining ability and heterosis following Griffing's diallel Method II Model I. A broad spectrum of genetic variation among the six parents and fifteen F_1 hybrids was confirmed by analysis of variance. $(H_1/D)^{0.5}$ value indicated that partial dominance gene action controlled all the traits except capsaicin and total phenolic content. Based on general combining ability (GCA) results, parent P_3 (PLP-2s) was the best general combiner for all the traits except K and Na, followed by the parents P_6 (BU Capsicum 1), P_5 (Morich-8), P_4 (Chili Japan) and P_1 (Red Chili). Specific combining ability (SCA), along with heterotic response, revealed that the F_1 hybrid $P_3 \times P_6$ (PLP-2s × BU Capsicum 1) was the best hybrid, followed by the hybrids $P_4 \times P_6$ (Chili Japan × BU Capsicum 1) and $P_3 \times P_4$ (PLP-2s × Chili Japan), as they exhibited superiority for major nutritional components, such as capsaicin and ascorbic acid. Ultimately, the subsequent selection of the F_1 hybrids would help develop better nutritional-quality hybrids.

Keywords: Combining ability; functional food, heterosis; hybrid; phytochemicals

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Introduction

Chili is a healthy spice crop belonging to the family Solanaceae, subfamily Solanoideae and tribe Capsiceae. It is imperatively used in almost every cuisine in Bangladesh and other South Asian countries due to its pungent taste, attractive color, and flavor [1]. Besides its culinary use, it has nutritional and medicinal properties [2]. Chili is a great source of dietary phytochemicals, such as capsaicin, phenolics, flavonoids, carotenoids, oleoresin, chlorophyll, saponins, cyanogenic glycosides, stilbenes, tannins, nitrogenous compounds (alkaloids, amines, betalains), terpenoids, and other endogenous metabolites [3]. Capsaicin ($C_{18}H_{27}0_3$) is the main nutritional substance found in chili. It is a crystalline acrid volatile alkaloid compound that can decrease free radicals [4-6]. Capsaicin can reduce the risk of obesity and type II diabetes; it prevents sinusitis, stomach ulcers, and the spread of prostate cancer. It is also useful for the remission of headaches and physical pain and for reducing the risk of cardiovascular diseases and arthritis [7,8]. Chili contains a large amount of vitamin C (ascorbic acid) and other vitamins, particularly vitamin A, vitamin B_3 (niacin), vitamin B_6 (pyridoxine), vitamin B_9 (folate), vitamin E (α-Tocopherol), and vitamin K. It also contains a range of minerals, namely K, Ca, Mg, Na, P, Fe, Cu, Mn, and Zn [9].

Though it has many nutritional and medicinal properties, very few studies have been conducted to improve its quality traits. Knowing the nature and extent of genetic variability among the germplasm is crucial to help identify desirable parents for an efficient breeding program. A broad range of genetic variability exists in the chili landraces that are currently cultivated in Bangladesh. The desired nutritional traits of variable indigenous and exotic genotypes must be combined in a single genotype to develop varieties of increased nutritional quality. In Bangladesh, few attempts have been made to establish chili varieties, and their breeding attempt for nutritionally rich chili varieties is lacking. To date, only six chili varieties have been developed in Bangladesh. Among these six varieties, four varieties, namely BARI Morich-1, BARI Morich-2, BARI Morich-3, and BARI Mistimorich-1, were released by Bangladesh Agricultural Research Institute [10]; one chili variety, BU Capsicum 1, has been developed by Bangabandhu Sheikh Mujibur Rahman Agricultural University [11]; and one chili variety, Binamorich-1, has been introduced by Bangladesh Institute of Nuclear Agriculture [12]. Of the six varieties, only Binamorich-1 is rich in vitamins A and C. This study aimed to exploit valuable genetic constituents of six diverse parents to improve nutritional phytochemicals by selecting desirable parents and superior F_1 hybrids based on combining ability and the magnitude of both mid-parent and better-parent heterosis.

Experimental

Plant materials

The genetic variability of twenty diverse chili genotypes was studied, and based on the desired traits, six genotypes were selected [9]. These six genotypes were used as parents (P_1: Red Chili, P_2: Chili Padi, P_3: PLP-2s, P_4: Chili Japan, P_5: Morich-8, P_6: BU Capsicum 1) for crossing to produce F_1s (6x6 half diallel population) by following Griffing's diallel Method II [13]. In our previous study in

Table 1. Analysis of variance (ANOVA) for nutritional traits in a 6×6 half diallel population of chili.

Nutritional traits†	Replication (df 2)	Genotypes (df 20)	Parents (df 5)	F_1 (df 14)	Parents vs F_1 (df 1)	Error (df 40)
CAP	0.00	0.01**	0.01**	0.01**	0.01**	0.00
AAC	18.57**	1018.45**	669.18**	1184.10**	445.70**	1.84
AOC	2.66	1041.29**	1098.72**	951.96**	2004.66**	8.59
BCC	0.00	0.0395**	0.0803**	0.0232**	0.0631**	0.00
Chl a	0.001**	0.075**	0.101**	0.068**	0.034**	0.00
Chl b	0.001**	0.02**	0.03**	0.02**	0.002**	0.00
TCC	0.0003**	0.01**	0.01**	0.01**	0.004**	0.00
TPC	21.00**	135263.20**	113103.80 **	151533.20**	18280.52**	0.00
TFC	49.00	324097.40	147641.80	405868.20	61584.31	0.00
TAC	0.01	1.13	0.41	1.45	0.35	0.00
K	0.002**	0.03**	0.04**	0.02**	0.05**	0.00
Mg	0.01	0.02	0.02	0.02	0.06	0.01
Ca	0.00	0.14	0.31	0.07	0.34	0.00
Na	0.00	0.01**	0.02**	0.004**	0.002**	0.00

**significance at $p < 0.01$; The values presented in the parentheses are degrees of freedom. †CAP: Capsaicin content (%); AAC: Ascorbic acid content (mg/100g); AOC: Antioxidant capacity (µg/g FW); BCC: β - carotene content (mg/100g); Chl a: Chlorophyll a content (mg/g); Chl b: Chlorophyll b content (mg/g); TCC: Total carotenoid content (mg/g); TPC: Total phenolic content (µg/g FW); TFC: Total flavonoids content (µg/g FW); TAC: Total anthocyanin content (µg/g FW); K: Potassium content (%); Mg: Magnesium content (%); Ca: Calcium content (%); Na: Sodium content (%).

2017, these six parents and fifteen F_1 hybrids were used to study combining ability and heterosis for yield and yield-related characteristics [14]. This study collected samples from the same parents and fifteen F_1 hybrids in the same year (2017) to study combining ability and heterosis for nutritional phytochemicals. Two nearly symmetrical mature fruits (green and red) were collected from five randomly selected chili plants from each genotype in three replications (six parents and fifteen hybrids). Part of the freshly harvested mature green fruits was transferred to the laboratory for phytochemical analysis. The rest of the harvested green fruits were stored at -20°C for further investigation, and these fruits were used within a week to avoid any chance of quality deterioration. Mature red fruits were oven-dried, ground, and stored in an airtight polythene bag for future use.

Biochemical Analysis for Nutritional Phytochemicals

The capsaicin content (CAP) of chili was estimated by following the colorimetric method described by Das et al. [15] using a spectrophotometer (PD-303UV, APEL, Japan). The ascorbic acid content (AAC) was measured following the method provided by Oberbacher and Vines [16]. The antioxidant capacity (AOC) of chili fruit was measured by DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging assay following the procedure described by Zellama et al. [17]. Total β-carotene content (BCC) was measured by following the method described by Prasad et al. [18] using a spectrophotometer (PD-303UV, APEL, Japan). The chili samples for measuring total carotenoid content (TCC) and chlorophyll content (Chl a and Chl b) were prepared via the González-Cortés et al. [19] method. TCC, Chl a, and Chl b were quantified following the formulas provided by Aslam et al. [20]. Chili fruit's total phenolic content (TPC) was determined by following the Folin-Ciocalteu method [17].

Total flavonoid content (TFC) was determined spectrophotometrically by following the method described by Sarkar et al. [21]. A calibration curve was prepared from the standards, and the TFC (μg/g fresh weight) was expressed as catechin equivalent. For measuring total anthocyanin content (TAC), the chili samples were prepared following the protocol described by Lim et al. [22]. TAC (μg/g fresh weight) was calculated as cyanidin-3-O-glucoside equivalent using the absorbance and a molar extinction coefficient for anthocyanin at 530 nm of 30000 $L^{-1}M^{-1}cm^{-1}$ [23]. Potassium (K), magnesium (Mg), calcium (Ca), and sodium (Na) contents were determined by following the procedures provided by Piper [24] using an atomic absorption spectrophotometer (Model 200-30, Hitachi, Japan).

Statistical Analysis

Computer software Statistical Tools for Agricultural Research (STAR) was used to analyze variance (ANOVA). Mean, standard error (SE), coefficient of variation (CV), and Tukey's honest significant difference (HSD) for different nutritional phytochemicals were computed. Combining ability analysis was done by following the Diallel Method II Model I of Griffing [13] via the Diallel-R program in R software version 2.11.1. Griffing's analysis was selected with the intention to determine the parents' performance and relative contribution to the F_1s, as determined by the general and specific combining abilities. The statistical model of Method II Model I of Griffing [13] for the combining ability analysis has been described below:

$$x_{ij} = u + g_i + g_j + s_{ij} + \frac{1}{bc}\sum_k \sum_l e_{ijkl}$$

where, i,j = 1 …… p (p = no. of parents)
k = 1 …… b (b = no. of blocks/ replications)
l = 1 …… c (c = no. of observation in each plot)

x_{ij} is the mean of x_{ij} th genotype across k and l; u is the population mean; g_i is the GCA effect; s_{ij} is the SCA effect such that $S_{ij} = S_{ji}$ and e_{ijkl} is the environmental effect specific to ijkl th observation. Restrictions imposed are $\sum_i g_i = 0$ and $\sum_j s_{ij} + s_{ii} = 0$ (for each i).

The sum of squares (SS) was calculated as follows:

$$SS_g = \frac{1}{p+2}\left\{\sum_i (X_{i.} + x_{ii})^2 - \frac{4}{p}X_{..}^2\right\}$$

$$SS_s = \sum_{i\leq j}\sum x_{ij}^2 - \frac{1}{p+2}\sum_i (X_{i.} + x_{ii})^2 + \frac{2}{(p+1)(p+2)}X_{..}^2$$

where SS_g = sum of squares due to GCA
SS_s = sum of squares due to SCA
$X_{i.}$ = mean of the i^{th} parent
x_{ii} = mean value of i^{th} parent

X.. = mean of the $\frac{p(p-1)}{2}$ crosses and parental values

The effects were calculated as follows:

$$u = \frac{2}{p(p+1)}X_{..}$$

$$g_i = \frac{1}{p+2}\left[X_{i.} + x_{ii} - \frac{2}{p}X_{..}\right]$$

$$s_{ij} = x_{ij} - \frac{1}{p+2}\left[X_{i.} + x_{ii} + X_{j.} + x_{jj}\right] + \frac{2}{(p+1)(p+2)}X_{..}$$

Standard errors of effects were calculated as follows:

$$SE(g_i) = \left[\frac{(p-1)\,\sigma_e^2}{p(p+2)}\right]^{1/2}$$

$$SE(s_{ij}) = \left[\frac{(p^2+p+2)\,\sigma_e^2}{(p+1)(p+2)}\right]^{1/2} \qquad (i \neq j)$$

Mid-parent heterosis and better-parent heterosis were computed for all the nutritional traits following the formulae described by Tyagi et al. [25].

$$\text{Mid parent heterosis (MPH)} = \frac{\overline{F_1} - \overline{MP}}{\overline{MP}} \times 100$$

where $\overline{F_1}$ = Mean performance of F_1
$\overline{MP}$ = Mean performance of the mid parent

Better parent heterosis (BPH) or heterobeltiosis

$$= \frac{\overline{F_1} - \overline{BP}}{\overline{BP}} \times 100$$

where $\overline{F_1}$ = Mean performance of F_1
$\overline{BP}$ = Mean performance of the better parent

Table 2. Analysis of variance (ANOVA) of combining ability for nutritional traits in a 6×6 half diallel population of chili.

Source of variation†	df	Nutritional traits‡									
		CAP	AAC	AOC	BCC	Chl a	Chl b	TCC	TPC	K	Na
GCA	5	0.005**	431.49**	401.09**	0.02**	0.03**	0.01**	0.01**	53129.90**	0.02**	0.005**
SCA	15	0.003**	308.81**	185.10**	0.01**	0.02**	0.01**	0.001**	20961.24**	0.01**	0.002**
Error	40	0.00	0.61	2.86	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Genetic parameters§											
σ^2g		0.0002	15.33	27.00	15.33	0.001	0.001	0.0005	4021.08	0.001	0.0004
σ^2s		0.003	308.20	398.23	308.20	0.02	0.01	0.001	53129.90	0.01	0.002
σ^2g/σ^2s		0.07	0.05	0.07	0.05	0.04	0.19	0.33	0.08	0.13	0.18
$(H_1/D)^{0.5}$		1.32	0.81	0.74	0.65	0.81	0.92	0.56	1.11	0.54	0.62
h^2_n		0.33	0.61	0.64	0.71	0.61	0.55	0.75	0.45	0.78	0.70

**significance at $p < 0.01$. †GCA: General combining ability; SCA: Specific combining ability. ‡CAP: Capsaicin content (%); AAC: Ascorbic acid content (mg/100g); AOC: Antioxidant capacity (µg/g FW); BCC: β - carotene content (mg/100g); Chl a: Chlorophyll a content (mg/g); Chl b: Chlorophyll b content (mg/g); TCC: Total carotenoid content (mg/g); TPC: Total phenolic content (µg/g FW); K: Potassium content (%); Na: Sodium content (%). §σ^2g: General combining ability variance; σ^2s: Specific combining ability variance; $(H_1/D)^{0.5}$: Degree of dominance; h^2_n: Heritability in narrow sense.

Table 3. General combining ability (GCA) effects for nutritional traits in 6×6 half diallel population of chili.

Parents	Nutritional traits†									
	CAP	AAC	AOC	BCC	Chl a	Chl b	TCC	TPC	K	Na
P_1	-0.02**	-10.35**	6.53**	-0.02**	0.05**	0.01**	0.004	-95.50**	0.01**	-0.02**
P_2	-0.001**	-4.23**	-0.19	-0.07**	0.08**	0.05**	0.02**	-1.82**	0.00	0.03**
P_3	0.02**	8.80**	4.53**	0.08**	0.03**	0.01**	0.03**	0.84**	-0.01**	-0.01**
P_4	0.02**	-2.63**	-6.08**	0.00	-0.09**	-0.06**	-0.01**	34.63**	0.05**	0.02**
P_5	0.02**	0.67*	-0.99	-0.02**	-0.04**	0.03**	-0.03**	51.71**	-0.08**	-0.03**
P_6	-0.04**	7.74**	-3.80**	0.03**	-0.02**	-0.04**	-0.02**	10.13**	0.04**	0.01**
$SE(g_i)$	0.0001	0.25	0.55	0.002	0.001	0.002	0.001	0.001	0.00	0.00
$SE(g_i-g_j)$	0.0002	0.39	0.85	0.003	0.002	0.002	0.003	0.001	0.00	0.00

* and ** significance at 5% and 1% levels, respectively. †CAP: Capsaicin content (%); AAC: Ascorbic acid content (mg/100g); AOC: Antioxidant capacity (µg/g FW); BCC: β - carotene content (mg/100g); Chl a: Chlorophyll a content (mg/g); Chl b: Chlorophyll b content (mg/g); TCC: Total carotenoid content (mg/g); TPC: Total phenolic contents (µg/g FW); K: Potassium content (%); Na: Sodium content (%). P_1: Red Chili, P_2: Chili Padi, P_3: PLP-2s, P_4: Chili Japan, P_5: Morich-8, P_6: BU Capsicum 1.

Results

Genetic Parameters Analysis for Nutritional Traits

Simple ANOVA indicated that variations attributable to the parents and F_1s were significant for all the nutritional phytochemicals except TAC, TFC, Ca, and Mg contents (Table 1). The subsequent analyses did not include the non-significant traits (Table 1). The combining ability analysis indicated that variation attributable to GCA and SCA was highly significant for the nutritional phytochemicals. General combining ability variance (σ^2g) was highest for TPC, which indicated the predominance of additive gene effects for this trait (Table 2). The high specific combining ability variance (σ^2s) for AAC, AOC, BCC, and TPC revealed that non-additive gene effects played a significant role in the inheritance of these traits. σ^2g was lower than σ^2s for all the phytochemicals studied, and the lower σ^2g/σ^2s ratio (<1) was also found for all the nutritional phytochemicals that revealed non-additive gene action for all the nutritional characters. The $(H_1/D)^{0.5}$ ratio indicates that the degree of dominance was greater than zero but less than one for all the nutritional traits except CAP (1.32) and TPC (1.11), and the heritability in the narrow sense (h^2_n) was high (>0.5) for all the traits except CAP (0.33) and TPC (0.45) (Table 2).

Analysis of Combining Ability for Nutritional Traits

The estimation of GCA effects revealed that the parents P_3, P_4, and P_5 showed the highest and most significant positive value for CAP among the parents (Table 3). The most significant positive GCA effects for AAC and BCC were found in parent P_3 followed by parent P_6. For AOC,

Table 4. Specific combining (SCA) ability effects for nutritional traits in 6×6 half diallel population of chili.

Crosses	Nutritional traits†									
	CAP	AAC	AOC	BCC	Chl a	Chl b	TCC	TPC	K	Na
$P_1 \times P_2$	-0.004**	16.64**	-20.13**	0.08**	-0.02**	-0.06**	0.05**	-22.37**	0.01**	-0.05**
$P_1 \times P_3$	-0.04**	-9.39**	-12.30**	-0.15**	-0.17**	-0.09**	-0.05**	-277.50**	-0.15**	0.08**
$P_1 \times P_4$	0.05**	5.58**	2.00	-0.09**	-0.08**	-0.06**	-0.05**	37.72**	-0.06**	0.01**
$P_1 \times P_5$	0.004**	-5.85**	23.64**	0.03**	-0.05**	0.01**	0.00	-16.49**	0.04**	0.03**
$P_1 \times P_6$	-0.02**	-8.36**	-30.22**	0.004	-0.19**	-0.09**	-0.01	239.95**	-0.01**	0.01**
$P_2 \times P_3$	0.001**	-32.81**	-12.59**	-0.09**	-0.02**	-0.02**	-0.01	-3.86**	0.003**	0.06**
$P_2 \times P_4$	-0.003**	-9.24**	18.76**	-0.05**	-0.16**	-0.01	-0.03**	60.37**	0.01**	-0.06**
$P_2 \times P_5$	-0.005**	5.20**	-25.45**	0.04**	-0.04**	0.08**	-0.06**	70.02**	-0.02**	-0.01**
$P_2 \times P_6$	-0.04**	7.23**	-4.94**	0.02**	0.34**	0.15**	0.01*	-219.58**	-0.15**	0.01**
$P_3 \times P_4$	0.07**	16.56**	-26.28**	0.06**	0.17**	0.06**	0.02**	-128.92**	-0.09**	0.01**
$P_3 \times P_5$	0.11**	10.20**	-8.25**	-0.05**	0.10**	0.04**	0.07**	517.36**	0.06**	-0.01**
$P_3 \times P_6$	0.03**	11.99**	29.92**	0.17**	-0.14**	-0.04**	0.01*	141.61**	-0.06**	0.02**
$P_4 \times P_5$	-0.05**	-40.13**	9.05**	-0.01*	-0.01**	-0.02**	-0.01*	-282.76**	0.08**	-0.02**
$P_4 \times P_6$	0.07**	13.90**	1.92	0.17**	0.06**	0.03**	0.002	-288.71**	0.06**	-0.04**
$P_5 \times P_6$	-0.05	-6.74	1.35	-0.09	-0.01	-0.05	-0.01	11.54	-0.10	0.05
$SE(s_{ii})$	0.0003	0.57	1.24	0.004	0.003	0.003	0.004	0.002	0.00	0.00
$SE(s_{ij})$	0.0004	0.69	1.50	0.01	0.004	0.004	0.01	0.002	0.00	0.00
$SE(s_{ii}\text{-}s_{jj})$	0.0004	0.78	1.69	0.01	0.01	0.005	0.01	0.002	0.00	0.00
$SE(s_{ij}\text{-}s_{ik})$	0.001	1.04	2.24	0.01	0.01	0.01	0.01	0.003	0.00	0.00
$SE(s_{ij}\text{-}s_{kl})$	0.001	0.96	2.07	0.01	0.01	0.01	0.01	0.003	0.00	0.00

* and ** significance at 5% and 1% levels, respectively. †CAP: Capsaicin content (%); AAC: Ascorbic acid content (mg/100g); AOC: Antioxidant capacity (µg/g FW); BCC: β - carotene content (mg/100g); Chl a: Chlorophyll a content (mg/g); Chl b: Chlorophyll b content (mg/g); TCC: Total carotenoid content (mg/g); TPC: Total phenolic contents (µg/g FW); K: Potassium content (%); Na: Sodium content (%). P_1: Red Chili, P_2: Chili Padi, P_3: PLP-2s, P_4: Chili Japan, P_5: Morich-8, P_6: BU Capsicum 1.

the most substantial positive GCA effects were observed in parent P_1 followed by parent P_3. The most significant positive GCA effects were observed in the parent P_2 for Chl a and Chl b. For TCC, parent P_3 showed the highest GCA effects, followed by parent P_2. Parent P_5 had the most significant positive GCA effects for TPC, followed by parents P_4, P_6, and P_3. The most substantial positive GCA effects for K were recorded in parent P_4, parents P6, and P1; parent P_2 showed the highest GCA effect for Na followed by parents P_4 and P_6.

The estimation of SCA (Table 4) of F_1 hybrids for nutritional phytochemicals revealed that for CAP, the hybrids $P_3 \times P_5$ exhibited the highest SCA following the hybrids $P_3 \times P_4$, $P_4 \times P_6$, $P_1 \times P_4$, and $P_3 \times P_6$. For AAC, the hybrid $P_1 \times P_2$ had maximum SCA followed by $P_3 \times P_4$, $P_4 \times P_6$, $P_3 \times P_6$, and $P_3 \times P_5$. The hybrid $P_3 \times P_6$ exhibited the maximum and significant positive SCA for AOC, followed by the hybrids $P_1 \times P_5$, $P_2 \times P_4$, and $P_4 \times P_5$. The higher SCA for BCC was found in the hybrids $P_4 \times P_6$ and $P_3 \times P_6$. Besides significant positive SCA, the hybrids $P_2 \times P_6$ contained the highest amount of Chl a followed by $P_3 \times P_4$ and $P_3 \times P_5$. In the case of Chl b, the F_1 $P_2 \times P_6$ indicated the maximum significant and positive SCA. For TCC, the higher significant positive SCA were recorded in $P_3 \times P_5$, $P_1 \times P_2$, $P_3 \times P_4$, $P_2 \times P_6$, and $P_3 \times P_6$. The highest significant positive SCA TPC was observed in hybrids $P_3 \times P_5$, followed by $P_1 \times P_6$ and $P_3 \times P_6$. The hybrids $P_4 \times P_5$ showed the highest significant positive SCA for K, while the most effective positive SCA for Na was observed in the hybrid $P_1 \times P_3$.

Estimation of Heterosis for Nutritional Traits

The analysis of mean performance revealed the existence of suitable genetic variation among the six parental genotypes and fifteen F_1s for the nutritional traits that can be exploited through heterosis breeding (Table 5). The superiority of F_1 hybrids or the mid-parent (average or relative) heterosis and better-parent heterosis (heterobeltiosis) for nutritional phytochemicals are presented in Table 6 and Figures 1, 2 & 3, respectively.

The mid-parent heterosis (%) revealed all the F_1 hybrids except $P_2 \times P_5$, $P_2 \times P_6$, $P_4 \times P_5$, and $P_5 \times P_6$ had significant and positive relative heterosis for CAP. The higher significance and positive mid-parent heterosis for AAC were observed in the hybrids $P_1 \times P_2$, $P_4 \times P_6$, $P_3 \times P_6$, and $P_3 \times P_4$. The hybrids $P_1 \times P_5$ and $P_3 \times P_6$ exhibited significant positive relative heterosis for AOC, and the hybrids $P_4 \times P_6$,

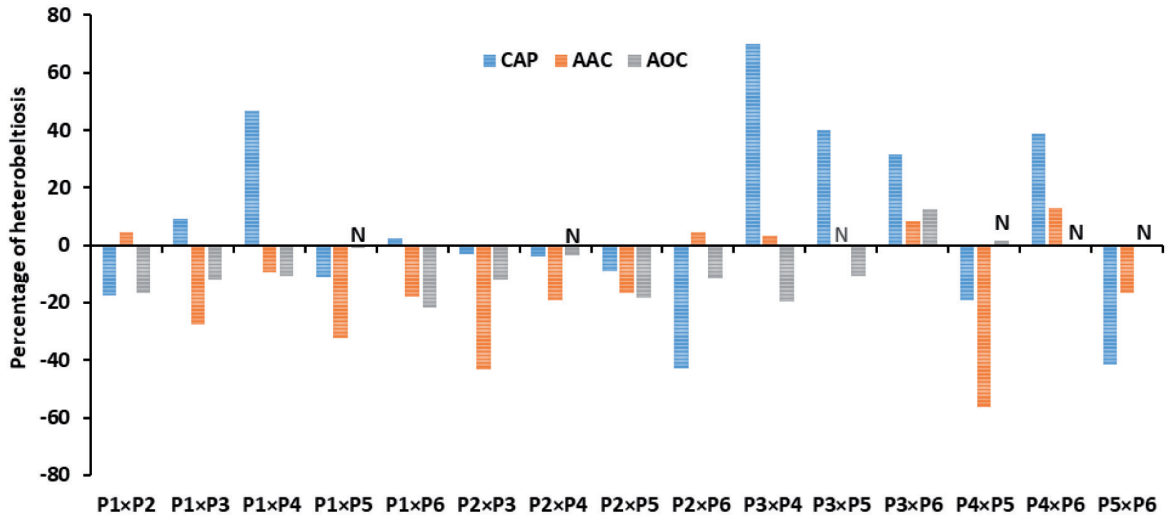

Fig. 1. Percentage of heterobeltiosis for CAP: Capsaicin content (%); AAC: Ascorbic acid content (mg/100g); AOC: Antioxidant capacity (µg/g FW). Non-significant data were marked by 'N' and other data were significant at P<0.01. P_1: Red Chili, P_2: Chili Padi, P_3: PLP-2s, P_4: Chili Japan, P_5: Morich-8, P_6: BU Capsicum 1.

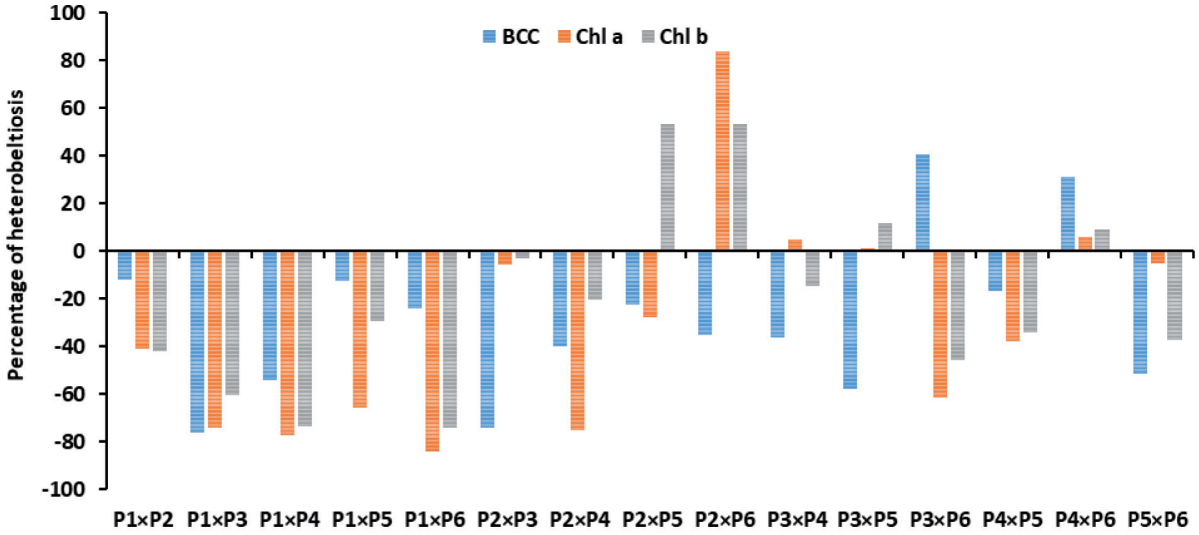

Fig. 2. Percentage of heterobeltiosis for BCC: β-carotene content (mg/100g); Chl a: Chlorophyll a content (mg/g); Chl b: Chlorophyll b content (mg/g). All the data were significant at P<0.01. P_1: Red Chili, P_2: Chili Padi, P_3: PLP-2s, P_4: Chili Japan, P_5: Morich-8, P_6: BU Capsicum 1.

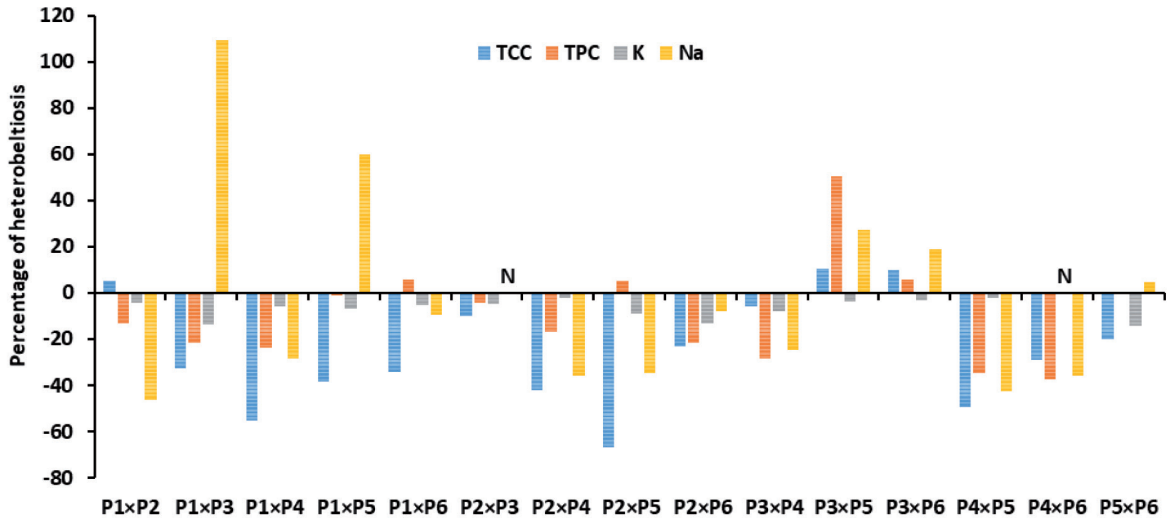

Fig. 3. Percentage of heterobeltiosis for TCC: Total carotenoid content (mg/g); TPC: Total phenolic content (µg/g FW); K: Potassium content (%); Na: Sodium content (%). Non-significant data were marked by 'N' and other data were significant at P<0.01. P_1: Red Chili, P_2: Chili Padi, P_3: PLP-2s, P_4: Chili Japan, P_5: Morich-8, P_6: BU Capsicum 1.

$P_3 \times P_6$, $P_1 \times P_2$, and $P_2 \times P_5$ had significant positive relative heterosis for BCC. The mid-parent heterosis for both Chl a and Chl b was positive and significant for the hybrids $P_2 \times P_6$, $P_3 \times P_4$, $P_3 \times P_5$, and $P_4 \times P_6$. The hybrids $P_3 \times P_5$, $P_3 \times P_6$, and $P_1 \times P_2$ showed higher significant relative heterosis for TCC, and the hybrids $P_3 \times P_5$, $P_1 \times P_6$, and $P_3 \times P_6$ exhibited significant positive relative heterosis for TPC. For K, the hybrids $P_4 \times P_5$, $P_3 \times P_5$, and $P_4 \times P_6$ expressed higher significant positive relative heterosis, and all the F_1's figured significant positive relative heterosis in the case of Na except $P_1 \times P_2$, $P_2 \times P_4$, $P_2 \times P_5$, $P_4 \times P_5$, and $P_4 \times P_6$ (Table 6).

The analysis of better parent heterosis or heterobeltiosis (%) exhibited that the hybrids $P_3 \times P_4$, $P_1 \times P_4$, $P_3 \times P_5$, $P_4 \times P_6$, and $P_3 \times P_6$ had higher significant positive heterobeltiosis for CAP. Significant and positive heterobeltiosis for AAC was observed in the hybrids $P_4 \times P_6$, $P_3 \times P_6$, $P_1 \times P_2$, $P_2 \times P_6$, and $P_3 \times P_4$, and only hybrids $P_3 \times P_6$ showed significant positive heterobeltiosis for AOC (Figure 1). For BCC, the hybrids $P_3 \times P_6$ and $P_4 \times P_6$ showed significant positive heterobeltiosis. The hybrids $P_2 \times P_6$, $P_4 \times P_6$, and $P_3 \times P_5$ exhibited significant positive heterobeltiosis for Chl a and Chl b (Figure 2). For TCC, the hybrids $P_3 \times P_5$, $P_3 \times P_6$, and $P_1 \times P_2$ had significant positive heterobeltiosis; and for TPC, it was shown in the hybrids $P_3 \times P_5$, $P_1 \times P_6$, $P_3 \times P_6$, and $P_2 \times P_5$. For Na, the hybrids $P_1 \times P_3$, $P_1 \times P_5$, $P_3 \times P_5$, $P_3 \times P_6$, and $P_5 \times P_6$ expressed significant positive heterobeltiosis (Figure 3).

Discussion

Genetic variance, gene action, and heritability information are used to select parents and hybrids. Simple ANOVA revealed the presence of a wide range of genetic variability among the parents and offspring, which can be used for developing hybrids for nutritional traits based on their combining ability and heterosis [26]. The ANOVA for combining ability manifested that the non-additive gene action caused the variability found for all the studied nutritional traits. The lower value of σ^2g/σ^2s ratio indicated that the non-additive gene action has prevailed for all the nutritional characteristics. As non-additive gene action is more important than additive gene action, there is a possibility of improving nutritional

Table 5. Mean performance of nutritional traits of six parents and fifteen F_1's in 6×6 half diallel population of chili.

Genotypes	Nutritional traits†									
	CAP	AAC	AOC	BCC	Chl a	Chl b	TCC	TPC	K	Na
P_1	0.15	70.00	271.34	0.26	0.61	0.38	0.18	1101.47	1.93	0.10
P_2	0.25	88.03	261.56	0.10	0.36	0.24	0.21	1327.21	1.91	0.26
P_3	0.18	109.33	263.59	0.58	0.34	0.26	0.19	1150.48	1.86	0.11
P_4	0.19	91.40	224.89	0.19	0.09	0.09	0.16	1643.55	1.93	0.28
P_5	0.27	110.00	237.63	0.24	0.18	0.24	0.08	1226.72	1.65	0.10
P_6	0.25	96.47	233.15	0.33	0.20	0.13	0.10	1350.98	1.97	0.21
$P_1 \times P_2$	0.21	92.07	225.98	0.23	0.36	0.22	0.22	1153.45	1.84	0.14
$P_1 \times P_3$	0.19	79.07	238.54	0.14	0.16	0.15	0.13	900.98	1.67	0.23
$P_1 \times P_4$	0.28	82.60	242.22	0.12	0.14	0.10	0.08	1249.99	1.82	0.20
$P_1 \times P_5$	0.24	74.47	268.97	0.23	0.21	0.27	0.11	1212.86	1.80	0.16
$P_1 \times P_6$	0.15	79.03	212.28	0.25	0.10	0.10	0.12	1427.71	1.86	0.19
$P_2 \times P_3$	0.24	61.77	231.52	0.15	0.34	0.25	0.19	1268.30	1.82	0.26
$P_2 \times P_4$	0.24	73.90	252.26	0.11	0.09	0.19	0.12	1366.32	1.89	0.18
$P_2 \times P_5$	0.24	91.63	213.15	0.19	0.26	0.37	0.07	1393.05	1.73	0.17
$P_2 \times P_6$	0.14	100.73	230.83	0.21	0.66	0.37	0.16	1061.87	1.71	0.24
$P_3 \times P_4$	0.33	112.73	211.94	0.37	0.36	0.22	0.18	1179.69	1.78	0.21
$P_3 \times P_5$	0.38	109.67	235.07	0.25	0.35	0.29	0.21	1843.05	1.80	0.14
$P_3 \times P_6$	0.23	118.53	270.42	0.17	0.13	0.14	0.17	1425.72	1.91	0.17
$P_4 \times P_5$	0.22	47.90	241.76	0.20	0.11	0.16	0.08	1076.72	1.89	0.16
$P_4 \times P_6$	0.27	109.00	231.81	0.43	0.21	0.14	0.11	1029.19	1.97	0.18
$P_5 \times P_6$	0.16	91.67	236.34	0.16	0.19	0.15	0.08	1346.52	1.69	0.22
CV (%)	0.00	1.51	1.22	4.15	3.06	3.79	6.91	0.00	0.00	0.00
SE	0.00	1.11	2.39	0.01	0.01	0.01	0.01	0.00	0.00	0.00
HSD (0.05)	0.00	4.22	9.14	0.03	0.02	0.03	0.03	0.01	0.00	0.00

CV: Coefficient of variation; SE: Standard error; HSD: Tukey's honest significant difference. †CAP: Capsaicin content (%); AAC: Ascorbic acid content (mg/100g); AOC: Antioxidant capacity (µg/g FW); BCC: β - carotene content (mg/100g); Chl a: Chlorophyll a content (mg/g); Chl b: Chlorophyll b content (mg/g); TCC: Total carotenoid content (mg/g); TPC: Total phenolic contents (µg/g FW); K: Potassium content (%); Na: Sodium content (%). P_1: Red Chili, P_2: Chili Padi, P_3: PLP-2s, P_4: Chili Japan, P_5: Morich-8, P_6: BU Capsicum 1.

Table 6. Mid parent heterosis for nutritional traits of fifteen F_1's in 6×6 half diallel population of chili.

Genotypes	Nutritional traits†									
	CAP	AAC	AOC	BCC	Chl a	Chl b	TCC	TPC	K	Na
$P_1 \times P_2$	2.76**	16.52**	-15.19**	25.97**	-25.52**	-28.79**	12.69**	-5.01**	-3.98**	-22.22**
$P_1 \times P_3$	17.46**	-11.82**	-10.81**	-66.87**	-66.53**	-52.94**	-30.71**	-19.98**	-12.06**	119.05**
$P_1 \times P_4$	64.73**	2.35*	-2.37	-46.75**	-60.09**	-56.79**	-52.78**	-8.93**	-5.65**	5.26**
$P_1 \times P_5$	13.58**	-17.26**	5.69*	-8.43**	-46.85**	-13.29**	-14.65**	4.19**	0.61**	60.00**
$P_1 \times P_6$	4.50**	-5.05**	-15.84**	-15.03**	-75.60**	-60.86**	-14.94**	16.43**	-4.47**	22.58**
$P_2 \times P_3$	14.24**	-37.41**	-11.83**	-55.94**	-3.14**	0.57**	-5.43**	2.38**	-3.47**	40.54**
$P_2 \times P_4$	8.33**	-17.63**	3.71	-22.21**	-59.98**	15.75**	-34.22**	-8.02**	-1.70**	-33.33**
$P_2 \times P_5$	-5.98**	-7.46**	-14.60**	7.83**	-4.26**	53.85**	-52.06**	9.09**	-2.45**	-5.56**
$P_2 \times P_6$	-27.48**	9.20**	-6.68**	-1.22**	136.70**	100.00**	4.34**	-20.70**	-11.79**	2.13**
$P_3 \times P_4$	78.31**	12.32**	-13.22**	-3.91**	67.32**	26.57**	1.99**	-15.56**	-6.32**	7.69**
$P_3 \times P_5$	69.33**	0.00	-6.20*	-39.98**	32.06**	15.95**	54.90**	55.06**	2.48**	33.33**
$P_3 \times P_6$	44.11**	15.19**	8.88**	62.50**	-51.29**	-27.51**	18.27**	13.99**	-0.57**	6.25**
$P_4 \times P_5$	-6.09**	-52.43**	4.54	-7.12**	-15.60**	-3.83**	-32.80**	-24.97**	5.49**	-15.79**
$P_4 \times P_6$	58.42**	16.04**	1.22	66.32**	47.26**	28.35**	-12.27**	-31.26**	1.12**	-26.53**
$P_5 \times P_6$	-24.15**	-11.20**	0.40	-43.97**	-1.31**	-17.98**	-11.90**	4.47**	-6.62**	41.94**

* and ** significance at 5% and 1% levels, respectively. †CAP: Capsaicin content (%); AAC: Ascorbic acid content (mg/100g); AOC: Antioxidant capacity (µg/g FW); BCC: β - carotene content (mg/100g); Chl a: Chlorophyll a content (mg/g); Chl b: Chlorophyll b content (mg/g); TCC: Total carotenoid content (mg/g); TPC: Total phenolic contents (µg/g FW); K: Potassium content (%); Na: Sodium content (%). P_1: Red Chili, P_2: Chili Padi, P_3: PLP-2s, P_4: Chili Japan, P_5: Morich-8, P_6: BU Capsicum 1.

traits by heterosis breeding in the later generation of segregating population [27]. Our study's non-additive gene action for CAP supported the results of previous studies by Aiswarya et al. [28] and Mahmood et al. [29]. In the case of AAC, we got the opposite effect that was reported by Aiswarya et al. [28], Do Nascimento et al. [30], and Tyagi et al. [25]. However, our findings of positive non-additive gene action for AAC and other phytochemicals may well contribute to the capture of genotypic potential to produce a useful amount of SCA potential in their progenies. The $(H_1/D)^{0.5}$ ratio measures the average degrees of dominance over all loci. $(H_1/D)^{0.5}$ was found to be greater than zero but less than one for AAC, AOC, BCC, Chl a, Chl b, TCC, K, and Na, indicating that these traits were controlled by partial dominance gene action, while the traits CAP and TPC were controlled by overdominance gene action. The h^2_n was high for AAC, AOC, BCC, Chl a, Chl b, TCC, K, and Na, indicating a significant part of additive gene action in phenotypic variability in nature, and selection would be effective for improvement of these traits in chili.

The estimation of GCA is essential for identifying suitable parents for developing hybrids with desired quality traits. It provides insight into whether a parent combines well in hybridization and indicates the specific performance of a hybrid against the parents' GCA expectations. Hybridization of parents with higher estimates of GCA should be potentially superior for the selection of lines in the advanced generations [31]. So far, in the present study, no parent was selected as an excellent general combiner for all the nutritional traits studied. Among the parents, the best GCA effect for all the traits except K and Na has been found in PLP-2s. Based on GCA effects, it is revealed that the parent Red Chili is the best parent for AOC, Chl a, Chl b, and K. Parent Red Chili could be selected for improving Chl a, Chl b, TCC, and Na in the hybrids. For developing hybrids with CAP, TPC, K, and Na, the parent Chili Japan; for CAP, AAC, Chl b, and TPC, the parent Morich-8 could be selected. The parent's BU Capsicum 1 could be a good general combiner for CAP, AAC, BCC, TPC, K, and Na. However, the parents PLP-2s, Morich-8, and BU Capsicum 1 could be considered for breeding hybrids for pungency and ascorbic acid content. The parents, Red Chili and Chili Padi, could be selected for developing sweet pepper with other phytochemicals as these parents showed negatively significant values for CAP.

Determination of SCA is important for identifying the best F_1 hybrids for specific traits. Both dominant and epistatic gene actions and heterosis can be indicated by SCA [32]. In the present study, the F_1 PLP-2s × BU Capsicum 1 is considered the best hybrid for improving major nutritional components such as capsaicin and ascorbic acid. The cross combination PLP-2s × Chili Japan and PLP-2s × Morich-8 could be selected for more CAP with higher AAC, Chl a, Chl b, and TCC. The combination PLP-2s × Chili Japan could also be considered for higher BCC and Na, and the hybrid PLP-2s × Morich-8 for higher TPC and K. The hybrid Chili Japan × BU Capsicum 1 could also be selected for CAP, AAC, BCC, Chl a, Chl b, and K. The hybrids mentioned above have combined most of the nutritional traits, and

these hybrids can be used for commercial exploitation. However, the other hybrids also performed well such as the hybrids Red Chili × Chili Padi could be selected for CAP, AAC, BCC, TCC, and K; and for CAP, AAC, TPC, and Na, the hybrid Red Chili × Chili Japan could be considered. The hybrid Red Chili × Morich-8 and Chili Padi × PLP-2s could improve CAP, K, and Na, and the hybrid Red chili × Morich-8 could also be considered for enhancing AOC, BCC, and Chl b. The hybrids Chili Padi × Chili Japan and Chili Japan × Morich-8 could be selected for lower CAP (can be cultivated as sweet pepper) with higher AOC and K. Additionally, the hybrid Chili Padi × Chili Japan was also well performed for TPC. For sweet peppers with higher AOC, BCC, and Chl b, the hybrids Chili Padi × Morich-8 and Chili Padi × BU Capsicum 1 could be selected. Simultaneously, the hybrid Chili Padi × Morich-8 could also be picked for higher TPC, and the hybrid Chili Padi × BU Capsicum 1 for Chl a, TCC, and Na.

The exploitation of heterosis is vital for developing hybrids with novel quality traits. Pungency (capsaicin) is considered the most important trait for the consumer in Bangladesh and some other countries in South Asia, and ascorbic acid is the second most important phytochemical in chili. However, we provide equal importance to combining capsaicin and other nutritional traits in the hybrids so that consumers can enjoy the spicy taste and additional nutrition. The nature and magnitude of heterobeltiosis help select superior hybrids and their exploitation to get better transgressive segregants [33]. The heterotic response of nutritional phytochemicals substantiated significant heterotic effects for all the nutritional characters studied [34]. The relative heterosis and heterobeltiosis indicate the potential for further improvement of the nutritional traits through heterosis breeding. Among the hybrids, 38% and 20% showed superiority over their mid-parent and better parent in the desired direction, considering the studied traits that reveal an outstanding potential for exploitable heterosis. All the nutritional traits for relative heterosis and all the characters except K for heterobeltiosis showed significant positive responses, which can be exploited in further breeding. Considering the overall SCA effects and both mid-parent heterosis and heterobeltiosis, the hybrid PLP-2s × BU Capsicum 1 could be selected as the best hybrid for CAP, AAC, AOC, BCC, TCC, TPC, and Na, followed by the hybrid Chili Japan × BU Capsicum 1 for CAP, AAC, BCC, Chl a, and Chl b; and the hybrid PLP-2s × Chili Japan for CAP, AAC, and Chl a. Other F_1 hybrids Red Chili × Chili Padi, Red Chili × Chili Japan, Red Chili × Morich-8, Chili Padi × BU Capsicum 1, and PLP-2s × Morich-8 could also be selected for different nutritional phytochemicals. Among them, the hybrid PLP-2s × BU Capsicum 1 has been chosen as the most suitable combination for breeding and selection in the next generations for commercial exploitation.

Conclusions

The result of the present study led to the identification of important parents based on GCA and positive SCA for developing F_1 hybrids with higher heterosis for chili nutritional phytochemicals. Parent PLP-2s was the best parent, followed by BU Capsicum 1, Chili Japan, and Morich-8, and the hybrids PLP-2s × BU Capsicum 1 was the best hybrid for all the nutritional phytochemicals except Chl a, Chl b, and K followed by Chili Japan × BU Capsicum 1 and PLP-2s ×P_4. Other F_1 hybrids, Red Chili × Chili Padi, Red Chili ×P_4, Red Chili × Morich-8, Chili Padi × BU Capsicum 1, and PLP-2s × Morich-8, showed heterosis for other nutritional quality. Overall, this study provides valuable information for genetic improvement of nutritional quality in chili through hybridization. Above hybrids also recommended for multilocation testing to assess their suitability for commercial cultivation.

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Conflict of Interest

The authors declare no conflict of interest.

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