CHILEAN JOURNAL of AGRICULTURAL RESEARCH

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RESEARCH ARTICLE

Enhancing traits in hot pepper through single cross-hybridization: A study of phenotypic and genotypic changes

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Received: 28 January 2024; Accepted: 28 April 2024, doi:10.4067/S0718-58392024000400476

ABSTRACT

The availability of hot pepper (*Capsicum frutescens* L.) hybrid varieties is limited compared to those in chili, bell or sweet pepper hybrid. Heterosis, heterobeltiosis, and reciprocal are critical indicators in F_1 hybrids, valuable for developing hybrid varieties. This research aimed to evaluate both phenotypic and genotypic changes in F_1 hybrids from single cross-hybridization. The study observed 53 phenotypic characteristics encompassing morphological (plant, leaves, flowers, and fruits), four production-related (fruit weight, fruit number per plant, fruit weight per plant, and yield per hectare), and 10 chemical characteristics (proximate, simple sugar, and secondary metabolite). Genotypic changes were analyzed using ten SSR markers. Employing a randomized complete block design, the study included two parents, F_1, and reciprocal F_1 hybrids, across four treatments with two replicates each. Phenotypic changes were analyzed using ANOVA and comparison test, while genotypic changes were analyzed through cluster analysis. Result indicated heterosis in 12 characters, heterobeltiosis in eight characters, and reciprocal effects in eight characters. Significant improvements were noted in key characteristics such as plant height, fruit maturity, fruit weight per plant, yield per hectare and vitamin C. Genotypic evaluation revealed distinct genetic differences between F_1 and its parents, including the reciprocal F_1. These phenotypic and genotypic changes suggest that hot pepper could be more productive and of higher quality when developed as a hybrid variety, indicating promising avenue for future breeding programs.

Key words: Better-parent heterosis, Euclidean, mid-parent heterosis, Tukey, UPGMA.

INTRODUCTION

Chili peppers (*Capsicum* spp.) consumed across the worldwide, have seen a global consumption surge to 40 million tons, a trend expected to continue rising with population growth (Tripodi et al., 2020; Parisi et al., 2020). Chili peppers are consumed in various forms, including fresh, dried (whole or as powder), and ground. They are also extensively used as a seasoning in various types of cuisine. Unique to chili peppers is capsaicin, a compound responsible for their spicy sensation, which affects taste perception. Beyond culinary, chili peppers serve as raw materials in the pharmaceutical industry with capsaicinoids recognized for their pharmacological properties

(Duranova et al., 2022). Their potential as anticarcinogenic, antidiabetic, anti-obesity, and anti-inflammatory agents' gamers a significant focus in health research (Otunola and Afolayan, 2015; Catalfamo et al., 2022; Mandal et al., 2023; Yasin et al., 2023).

Hot pepper (*Capsicum frutescens* L.) is one of the chili peppers varieties extensively cultivated by farmers in Indonesia. The productivity of hot pepper in Indonesia is around 8 t ha^{-1}, considerably low than the potential yield of 20 t ha^{-1} (Sahid et al., 2021; Syukur et al., 2022). This disparity is partly attributed to the predominant use of non-hybrid seeds. In Indonesia, hybrid hot pepper varieties make up only 25% of registered varieties in contrast to China, 80% hybrid cultivation, resulting in a productivity rate of 20 t ha^{-1} (Wei et al., 2020).

Several researches have reported the advantages of hybrid varieties over open-pollinated ones. Hybrids exhibit enhanced tolerance to abiotic stress, leading to higher yields (Wei et al., 2020). Singh et al. (2023) observed that heterosis or hybrid vigor could improve productivity, making the availability of hybrid varieties a potential solution to food and nutrition security amidst climate change. Heterosis plays a pivotal role in breeding for increased yield, quality improvement, and accelerated harvesting (Sharma et al., 2023). Hybrid seeds are a key component of chili cultivation technology that supports rural development and economic growth (Sayekti et al., 2020). The development of hybrids through heterosis can yield 30%-50% higher than open-pollinated cultivars (Nalla et al., 2021). Furthermore, the uniformity among individuals in hybrid varieties simplifies cultivation techniques and can reduce production costs (Rani et al., 2021).

Hybrid varieties generated through crossing activities between two pure lines, benefit significantly from reciprocal diallel crosses. This method involves alternating the male and female roles between two parent lines in breeding. Such crosses have shown notable improvement offspring production and quality compared to their parent lines. Syukur et al. (2023) reported that hybrids demonstrated higher α-glucosidase inhibition (AGI) activity and 2,2-diphenyl-1-picrylhydrazyl (DPPH) activities, surpassing their parent crosses. Similarly, Naves et al. (2022) found that hybrid chili varieties had greater capsaicinoid content than their parent varieties. Gomes et al. (2021) reported higher production in hybrids which can be influenced by the positioning of parents in the crossing process. This finding aligns with studies by do Nascimento et al. (2014) who also observed significant reciprocal effects on most morpho-agronomic traits and fruit quality.

Based on the background information, Indonesia currently faces a limited availability of hybrid hot pepper varieties, leading in farmers to rely predominantly on open-pollinated varieties. This research is designed to evaluate the performance of single-cross hybrids, including both parents, F_1 hybrid from the initial cross, and F_1 hybrid from reciprocal crosses. The aim of this research was to gain a deeper understanding of the advantages of hybrid hot peppers over their parents. It will also compare the outcome of reciprocal crosses and identify specific cross pairs with high yields and beneficial nutritional content. This study is expected to contribute valuable insight that could potentially enhance the cultivation and quality of hot pepper varieties in Indonesia, thereby impacting agricultural practices and outputs.

MATERIALS AND METHODS

Plant material and experimental design

This study utilized four *Capsicum frutescens* L. lines: Line A, line B, F_1 hybrids from the cross A×B (denoted as AB), and reciprocal F_1 hybrids from the cross B×A (denoted as BA). Lines A and B were late generation products of self-pollination, thus representing pure line. The experiment was conducted over a period spanning from July 2022 to August 2023. The location for the experiment was the Ministry of Agriculture research field, in the Lembang District (6°47'57'' S, 107°39'01'' E; 1250 m a.s.l.), West Bandung Regency, West Java Province, Indonesia. A randomized complete block design (RCBD) was employed for the experimental setup, with two replicates for each treatment. The experimental plot measured 1 m × 3 m. Each plot contained 12 plants summing up to a total experimental population of 96 plants.

Crop management

One month before planting, seeds were sown in trays with 107 holes. To enhance seed health and reduce the risk of fungal diseases, the seeds were pre-treated by soaking in a fungicide for 30 min. Seedling were maintained in a greenhouse to protect them from virus vector insects. One month after sowing, the seedlings were transplanted into the field. The field preparation was done simultaneously with seed sowing. The fertilization was applied per plot, including 6 kg chicken manure, 15 g dolomite, and 60 g NPK (16-16-16). To further support plant growth, an additional 5 g NPK (16-16-16) fertilizer per plant, was applied ten times at a 1 wk interval. Foliar fertilization was also applied monthly until the plants reached the harvest stage. Pest and disease control followed the methods described by Prabaningrum and Moekasan (2014) with modifications adapted to the field conditions.

Phenotypic evaluation

The evaluation encompassed 53 morphological characters, four production-related characters, and 10 chemical characters. Morphological characters were divided into 11 plant characters (anthocyanin coloration of hypocotyl, plant habit, stem length, stem width, shortened internode, number of internodes between the first flower and shortened internodes, internode length, anthocyanin coloration of nodes, intensity of anthocyanin coloration of nodes, hairiness of nodes, plant height), nine leaf characters (leaf length, leaf width, leaf intensity of green color, leaf anthocyanin coloration, leaf shape, leaf undulation of margin, leaf blistering, leaf profile in cross section, leaf glossiness), six flower characters (time of beginning of flowering, flower peduncle attitude, stigma exertion, anthocyanin coloration in anther, anthocyanin coloration in filament, secondary color of corolla), and 27 fruit characters (fruit color before maturity, fruit intensity of color before maturity, fruit anthocyanin coloration, fruit attitude, fruit length, fruit width, fruit ratio length/width, fruit shape in longitudinal section, fruit twisting, fruit shape in cross section, fruit sinuation of pericarp at basal part, fruit sinuation of pericarp excluding basal part, fruit texture of surface, fruit color at intermediate, fruit color at maturity, fruit intensity of color at maturity, fruit glossiness, fruit stalk cavity, fruit depth of stalk cavity, fruit shape of apex, fruit interlocular grooves, locules number, fruit wall thickness, stalk length, stalk width, calyx aspect, time of maturity).

Production characters included fruit weight per fruit (g), number of fruits per plant (fruits), fruit weight per plant (g), and fruit yield per plot (g). Morphological and production character samples were collected from the entire plant population. Experimental samples for chemical characters were composite fruit samples from 12 plants per lot, which were then dried into chili powder form. Chemical characters included water, ash, fat content, simple sugar (sucrose, fructose, glucose), total phenol, and total flavonoid as well as vitamin C and capsaicin (Valencia-Cordova et al., 2021; Aziz et al., 2021).

Genotypic evaluation

Genomic DNA was extracted from the young fresh leaves of chili peppers using a modified cetyl trimethyl ammonium bromide (CTAB) protocol (Aboul-Maaty and Oraby, 2019). The quantity of DNA stock solution was determined using a NanoDropTM2000 spectrophotometer (Thermo Scientific, Waltham, Massachusetts, USA), while the quality was performed by method on 1% agarose gel with 1× Tris-acetate-EDTA (TAE) buffer at 90 V for 30 min. The electrophoresis results were then visualized with a UV Transilluminator (UVP, Cambridge, UK). The genomic DNA of samples were subjected to PCR amplification using 10 SSR markers collected from some references as primers, namely AFF244121, CAMS152, CAMS211, CAMS362, CAMS420, CAMS806, CAMS839, HPMS1-62, HPMS1-148, HPMS1-173 (Nugroho et al., 2022). The PCR products were detected by staining them using ethidium bromide visualized under UV light using Transilluminator (BIO-RAD, Hercules, California, USA). The SSR marker was identified using standard molecular weight size markers (100 bp DNA ladder).

Data analysis

The mid-parent heterosis (MPH) was calculated using the formula 100 × ((F_1 - MP)/MP), where F_1 represents the average of the hybrid and MP is the mean of the parents. Better-parent heterosis (BPH) or heterobeltiosis was calculated using the formula: 100 × ((F_1 - BP)/BP), where F_1 is the hybrid average and BP is the mean of the better parent (Aiswarya et al., 2020; Naves et al., 2022). Reciprocal effects were calculated based on BPH-MPH. To determine differences between treatments, all data were analyzed using ANOVA and further tested using the Tukey test. Data analysis was performed using the PAST 3.1 (available at https://past.en.lo4d.com; Hammer et al., 2001) and PKBT 3.1 (Center for Tropical Horticulture Studies, IPB University, Bogor, Indonesia; available at http://pbstat.com/pkbt-stat/index.php) programs. For the genotypic character, the allele score was given based on the presence of a particular size allele in each sample using GelAnalyzer software (available at www.gelanalyzer.com) by Istvan Lazar Jr., PhD and Istvan Lazar Sr., PhD, CSc. Genetic parameters such as the number of alleles per locus, heterozygosity, gene diversity and the polymorphism information content (PIC) were estimated by PowerMarker V3.25 software (Bioinformatics Research Center, North Carolina State University, Raleigh, North Carolina, USA; https://brcwebportal.cos.ncsu.edu).

RESULTS

Plant characters

A total of 11 plant traits were recorded, comprising nine qualitative and four quantitative traits. No discernible distinctions were noted between the parental plants and the hybrid for the qualitative traits. Conversely, significant differences were observed between the two parental plants for quantitative traits such as stem length (A = 38.5 cm; B = 24.1 cm), stem width (A = 9.9 mm; B = 8.8 mm), internode length (A = 11.3 cm; B = 7.6 cm), and overall plant height (A = 101.5 cm; B = 92.6 cm). Heterosis was evident in stem length (3.2) and plant height (16.0), while heterobeltiosis effects were noted in plant height (11.0). Reciprocal effects were observed in stem length and stem width. The F_1 and reciprocal plant height (AB = 112.6 cm; BA = 117.4 cm) surpassed those of the parental (A = 101.5 cm; B = 92.6 cm) (Table 1, Figure 1).

Table 1. Means of parents (A and B), F_1 (AB), and reciprocal F_1 (BA) for hot pepper traits. Mid-parent heterosis (MPH) and best-parental heterosis (BPH). *Significant at p = 0.05 by the Tukey test. SHU: Scoville heat units.

Characters	A	B	AB	BA	MPH	BPH
Stem length, cm	38.5	24.1	32.3	36.2*	3.2*	-16.1
Stem width, mm	9.9	8.8	9.8	9.2	4.8	-0.7
Internode length, cm	11.3	7.6	10.7	10.4	12.5	-5.6
Plant height, cm	101.5	92.6	112.6*	117.4*	16.0*	11.0*
Leaf length, cm	13.4	12.4	13.8	14.3*	6.5*	2.8*
Leaf width, cm	6.4	5.3	6.5	6.2	11.5	1.8
Time of beginning of flowering, d after planting	70.3	71.2	70.9	69.1	0.3	-0.1
Fruit maturity, d after planting	128.0	136.5	124.9*	121.9*	-5.4*	-8.2*
Fruit length, cm	5.8	4.7	5.5	5.9*	3.0*	-5.9
Fruit width, mm	9.7	11.9	11.4	10.7	5.5	-4.3
Thickness of fruit flesh, mm	1.0	0.8	1.0	1.0	11.1	0.0
Stalk length, cm	3.1	3.2	3.1	3.1	-3.1	-3.1
Stalk thickness, mm	1.7	1.7	1.8	1.6	3.9	3.9
Fruit weight, g	2.9	1.4	2.6	3.7*	16.1*	-11.9
Fruit number per plant, nr	189.6	273.5	272.6	200.0	17.7	-0.3
Fruit weight per plant, g	552.2	329.7	695.8*	748.3*	57.8*	26.0*
Yield, t ha^{-1}	16.0	11.5	21.2*	21.6*	53.6*	32.5*
Water content, %	6.4	12.8	11.8	12.1	23.4	-7.2
Ash content, %	8.8	8.6	7.2*	9.3	-17.5*	-18.7*
Lipid content, %	4.6	4.4	6.8	5.0	51.8	49.5
Sucrose, mg g^{-1}	45.0	132.0	69.0	45.0	-22.0	-47.7
Glucose, mg g^{-1}	45.0	314.5	184.0	45.0*	2.3*	-41.5*
Fructose, mg g^{-1}	45.0	992.5	798.5	198.5*	53.9*	-19.5
Phenol, mg g^{-1}	186.3	222.7	212.4	211.3	3.9	-4.6
Flavonoid, mg g^{-1}	133.2	147.3	104.5	181.2	-25.5	-29.0
Vitamin C, mg 100 g^{-1}	96.9	47.6	144.8*	141.1*	100.3*	49.5*
Capsaicin, SHU	36923.9	42763.5	43720.5	58239.6	9.7	2.2

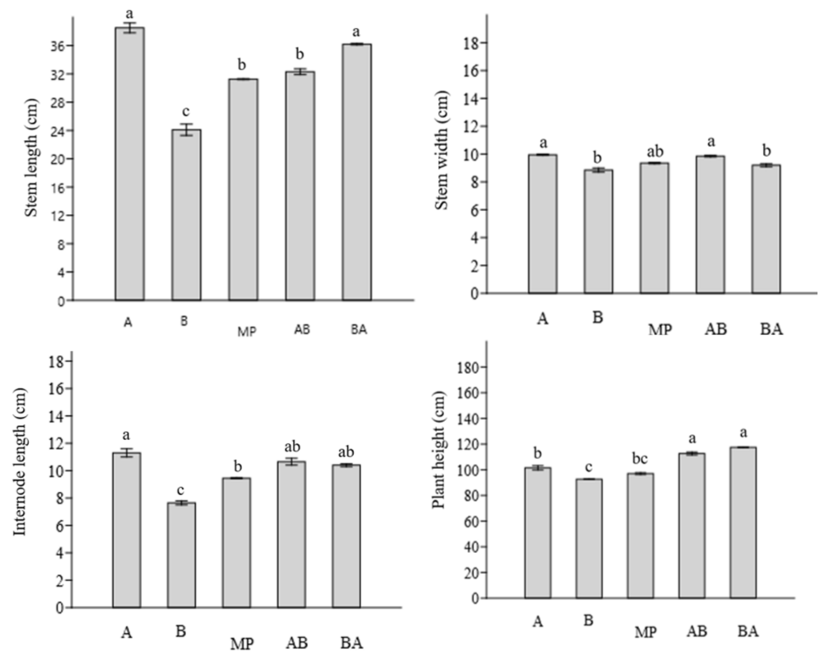

Figure 1. Plant characters performance of parents (A and B), the mean of the parents (MP), F_1 (AB), and reciprocal F_1 (BA). Different letters differ markedly based on the 5% Tukey test.

Leaf characters

A total of nine leaf characters were observed, including seven qualitative and two quantitative characters. Among these, a notable difference was found only in the undulation of the leaf margin. The undulation of leaf margin of parent A and parent B differed. Parent A had weak leaf margin undulation, while parent B displayed strong undulation. The F_1 hybrid and reciprocal F_1 hybrid both had medium undulation of the leaf margin, with F_1 appearing slightly stronger than the reciprocal F_1 (Figure 2). Heterosis and heterobeltiosis effects were observed in leaf length, where the length of the reciprocal F_1 leaf (14.3 cm) was longer and significantly different from two parents, respectively A: 13.4 cm and B: 12.4 cm. Reciprocal effect was not observed in quantitative leaf characters (Table 1, Figure 3).

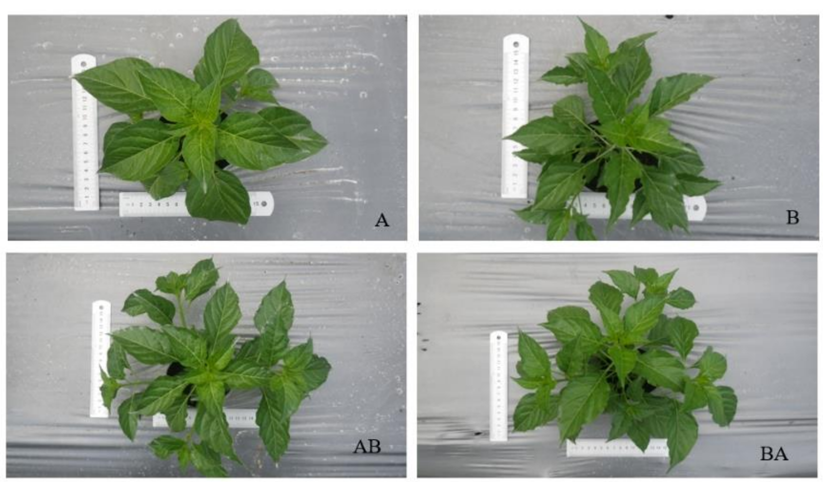

Figure 2. Performance of parent (A and B) and F_1 (AB and BA) leaves.

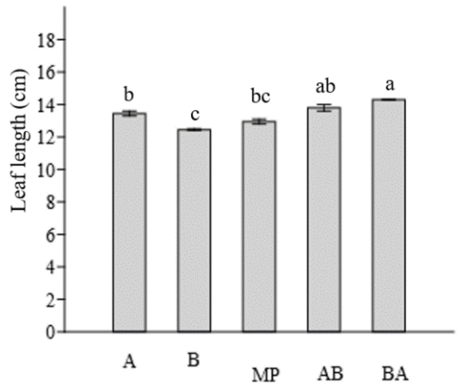

Figure 3. Leaf length of parents (A and B), the mean of the parents (MP), F_1 (AB), and reciprocal F_1 (BA). Different letters differ markedly based on the 5% Tukey test.

Flower and fruit characters

Six flower characters were observed, consisting of five qualitative and one quantitative character. All qualitative characters did not show differences between parents and the hybrid. Heterosis, heterobeltiosis, and reciprocal effects were not observed in flower characters.

A total of 27 fruit characters were observed, comprising 21 qualitative and six quantitative characters. Qualitatively, differences between parents and F_1 were observed in the fruit situation of the pericarp at the basal part and the number of fruits locules. In quantitative characters, both parents showed significant differences in fruit maturity (A = 128 d after planting and B = 137 d after planting), fruit length (A = 5.8 cm and B = 4.7 cm), and thickness of fruit flesh (A = 1.0 mm and B = 0.8 mm). No reciprocal effects were observed in quantitative fruit characters. Heterosis effects were observed in days to fruit maturity (-5.4) and fruit length (3.0). Heterobeltiosis effect was observed in days to fruit maturity (-8.2) (Table 1, Figure 4). The fruit performance of parents and hybrids is presented in Figure 5.

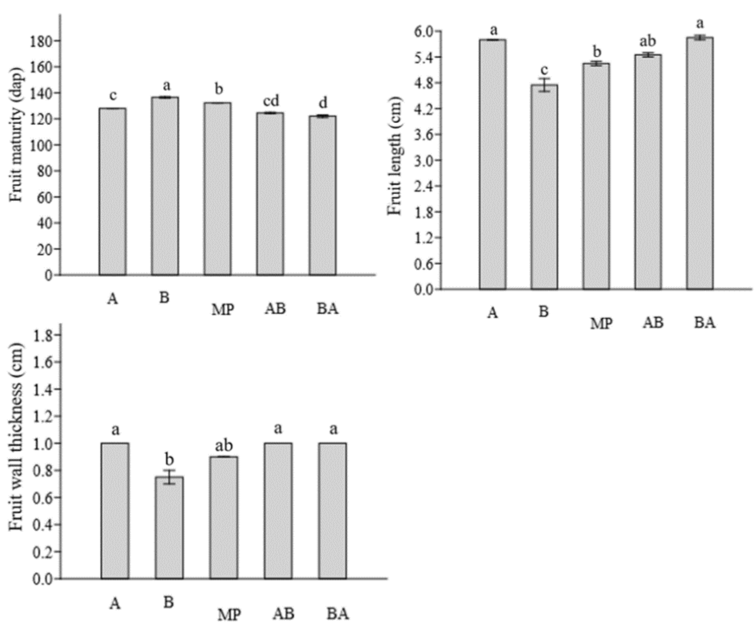

Figure 4. Fruit characters of parents (A and B), the mean of the parents (MP), F_1 (AB), and reciprocal F_1 (BA). dap: Days after planting. Different letters differ markedly based on the 5% Tukey test.

Figure 5. Performance of parental fruits (A and B) and F_1 (AB and BA).

Production-related characters

Four production-related characters were observed. Both parents showed significant differences in fruit weight (A = 2.9 g and B = 1.4 g), fruit weight per plant (A = 189.6 g and B = 273.5 g), and fruit yield per hectare (A = 16 t and B = 11.5 t). Heterosis was observed in fruit weight, fruit weight per plant, and yield per hectare. AB and BA had higher yield per hectare than their parents (AB = 21.2 t, BA = 21.6 t, A = 16 t and B = 11.5 t). Heterobeltiosis was observed in fruit weight per plant (26.0) and yield per hectare (32.5). There was reciprocal effect on fruit weight (AB = 2.6 g and BA = 3.7 g) (Table 1, Figure 6).

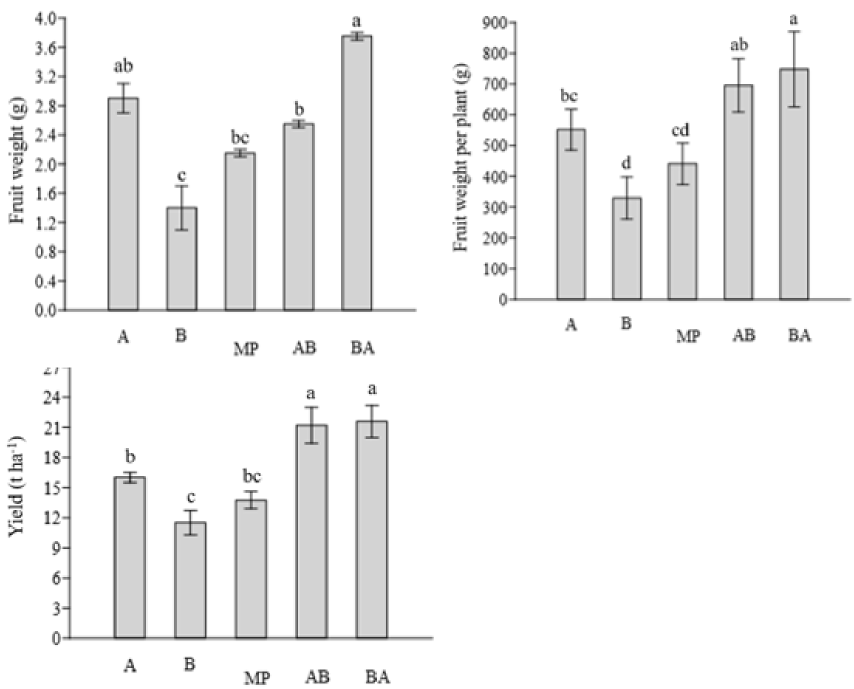

Figure 6. Production-related characters (A and B), the mean of the parents (MP), F_1 (AB), and reciprocal F_1 (BA). Different letters differ markedly based on the 5% Tukey test.

Chemical characters

Ten chemical characters were observed, categorized into three proximate, three simple sugar, and four secondary metabolite characters. In proximate characters, the only difference between the parents was in water content. The heterosis and heterobeltiosis effect occurred in ash content (Table 1, Figure 7). In terms of simple sugar characters, both parents show highly significant differences, negative heterosis and reciprocal effects in fructose (A = 45, B = 992.5, AB = 798.5 and BA = 198.5) (Table 1, Figure 8). Regarding secondary metabolite characters, both parents show significant differences in phenol (A = 186.3 and B = 222.7). Heterosis and heterobeltiosis effects were observed in vitamin C (A = 96.9, B = 47.6, AB = 144.8 and BA = 141.1). Reciprocal effects were observed in the flavonoid content (A = 133.2, B = 147.3, AB = 104.5 and BA = 181.2) (Table 1, Figure 9).

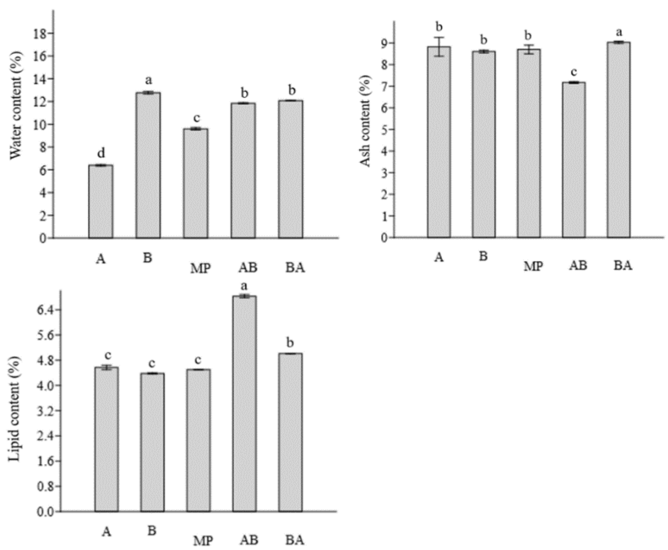

Figure 7. Proximate characters of parent (A and B), the mean of the parents (MP), F_1 (AB), and reciprocal F_1 (BA). Different letters differ markedly based on the 5% Tukey test.

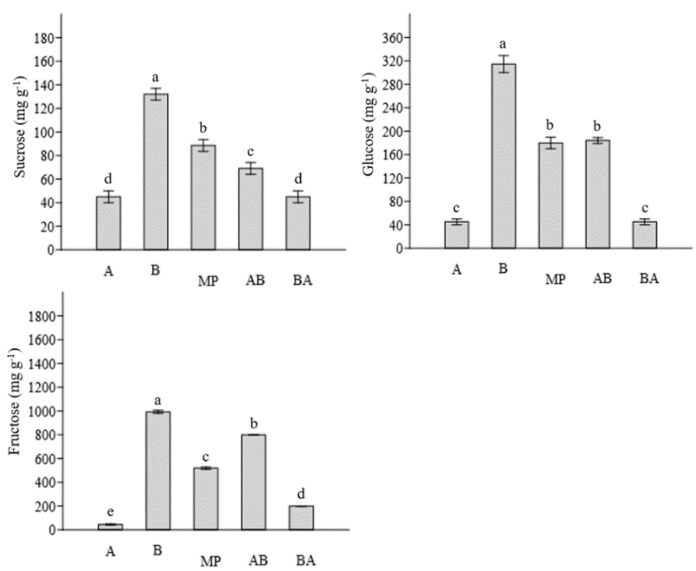

Figure 8. Simple sugar characters of parents (A and B), the mean of the parents (MP), F_1 (AB), and reciprocal F_1 (BA). Different letters differ markedly based on the 5% Tukey test.

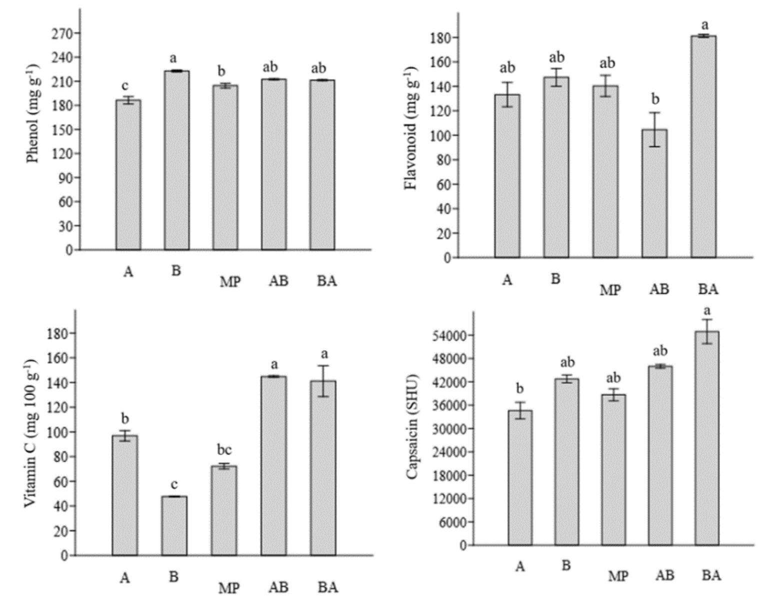

Figure 9. Secondary metabolites of parents (A and B), the mean of the parents (MP), F_1 (AB), and reciprocal F_1 (BA). Different letters differ markedly based on the 5% Tukey test. SHU: Scoville heat units.

Genotypic characteristics

Statistical information on SSR polymorphism observed in both parent and F_1 hybrids, providing crucial insights into genetic diversity and heterozygosity. The statistical summary of the polymorphism of 10 SSR markers is presented in Table 2. Marker analysis reveals varying frequencies of major alleles, ranging from 0.25 to 1.00. The number of alleles per marker ranges from 1.0 to 5.0, indicating the richness of genetic variants. Among the ten SSR markers, CAMS806 amplified more alleles than other markers. Gene diversity, depicted by the polymorphism information content (PIC), ranges from 0.0 to 0.78, highlighting the extent of genetic variability within the studied population. Overall, the mean statistics across markers provide a comprehensive view, indicating an average major allele frequency of 0.55, an average of 2.70 alleles per marker, a mean gene diversity of 0.51, and an average heterozygosity of 0.37, emphasizing the genetic richness and diversity present in both parent plants and their F_1 hybrids. The genetic similarity between F_1 progeny AB and parent B was typically higher (at 85%) compared to the reciprocal, where the similarity between F_1 progeny BA and parent A was 45% (Figure 10).

Table 2. Statistical information of SSR polymorphism observed in the parent's plants and the F_1 hybrids. PIC: Polymorphism information content.

Markers	Major allele frequency	Allele number	Gene diversity	Heterozygosity	PIC
AFF244121	0.5000	3.0000	0.6250	1.0000	0.5547
CAMS152	0.3750	3.0000	0.6563	0.7500	0.5815
CAMS211	0.3750	4.0000	0.6875	1.0000	0.6299
CAMS362	0.7500	2.0000	0.3750	0.0000	0.3047
CAMS420	1.0000	1.0000	0.0000	0.0000	0.0000
CAMS806	0.2500	5.0000	0.7813	0.7500	0.7456
CAMS839	0.7500	2.0000	0.3750	0.0000	0.3047
HPMS1-62	0.5000	2.0000	0.5000	0.0000	0.3750
HPMS1-148	0.5000	3.0000	0.5938	0.2500	0.5112
HPMS1-173	0.5000	2.0000	0.5000	0.0000	0.3750
Mean	0.5500	2.7000	0.5094	0.3750	0.4382

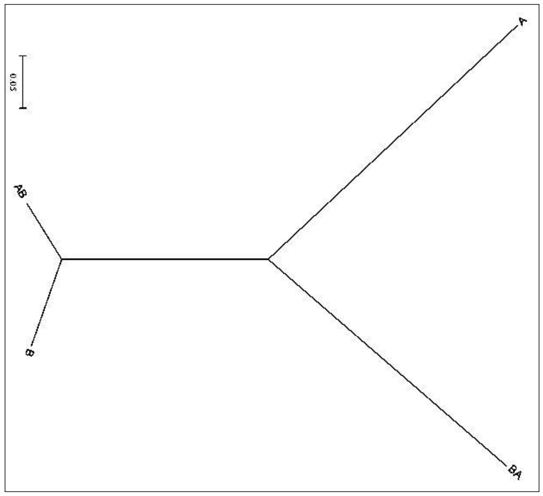

Figure 10. Dendrogram of genetic similarity among parents (A and B), F_1 (AB), and reciprocal F_1 (BA).

DISCUSSION

This research aimed to evaluated the performance of 53 single-cross hybrid traits in hot pepper, including their reciprocal crosses, examining heterosis, heterobeltiosis, and reciprocal effects. The cross between two parent lines was expected to have significant heterosis values. In this study, heterosis, heterobeltiosis, and reciprocal effects were observed in 12 characters (22.6%), 8 characters (15.1%), and 8 characters (15.1%) of the traits respectively (Table 1). Heterosis is defined as a condition in which the performance of the F_1 offspring significantly surpasses the mid-parent value (Karim et al., 2021; Naves et al., 2022; Singh et al., 2023). Twelve traits exhibiting heterosis included stem height, plant height, leaf length, fruit maturity, fruit length, fruit weight, fruit weight per plant, yield per hectare, ash content, fructose, glucose and vitamin C. The crossing of parent lines A and B resulted in offspring AB and BA with improved characters. The dominant genes of A and B that excel in performance have been consolidated in the genotypes AB and BA. The hybrids AB and BA exhibit improved characteristics such as plant height (cm) (A = 101.5, B = 92.6, AB = 112.6 and BA = 117.4), faster fruit maturity (days after planting) (A = 128 B = 136.5, AB = 124.9 and BA = 121.9), higher fruit weight per plant (g) (A = 552.2, B = 329.7, AB = 695.8 and BA = 748.3), higher fruit yield (t ha^{-1}) (A = 16, B = 11.5, AB = 21.2 and BA = 21.6), and higher chemical content especially vitamin C (mg 100 g^{-1}) (A = 96.9, B = 47.6, AB = 144.8 and BA = 141.1) and flavonoid (mg 100 g^{-1}) (A = 133.2, B = 147.3 and BA = 181.2).

Research on heterosis in hot pepper is still limited. Amorim et al. (2021) observed that heterosis in production-related traits of chili pepper (*C. frutescens*) but not in fruit length and the number of fruits per plant. This observation is consistent with the findings of this research, which also noted heterosis in fruit weight per plant and fruit weight per plot but not in fruit length and number of fruits per plant. Heterosis effects on yield-related traits and yield components have been widely reported in *C. annuum*. Devi and Sood (2018) reported positive heterosis for fruit length, number of fruits per plant, and fruit yield per plant. Aiswarya et al. (2020) found heterosis in nearly all quantitative fruit traits, including fruit weight per fruit, fruit weight per plant, and fruit yield per plot with heterosis in fruit yield per plot reaching up to 142%. Aditika et al. (2020) and Syukur et al. (2023) also reported significant heterosis effects on various yield traits. Chowdhury et al. (2023) reported the correlation between yield and yield component, the number of fruits per plant had a highly significant positive correlation with fruit yield per plant.

Heterosis in the chemical traits of chili peppers has been reported in several studies. The observed heterosis effect in capsaicin content in this research aligns with findings of Naves et al. (2022), who discovered heterosis effects on capsaicin accumulation in the placenta of hybrid fruits resulting from crossbreeding *C. chinense* and *C. annuum*. Contrary, Jaiswal et al. (2022), who reported higher levels of sucrose and fructose in parents compared to F_1 hybrids, this study reveals lower sucrose levels but higher fructose levels in F_1 hybrids. Furthermore, Aiswarya et al. (2020) reported that F_1 hybrids have higher levels of vitamin C and capsaicin compared to parents, a finding mirrored. In this study, particularly with vitamin C in F_1 hybrid being significantly higher than in parents, and receiving the highest heterosis value among all the evaluated quantitative traits. This suggest that the F_1 hybrids developed in this study could emerge as a new cayenne chili variety, distinguished by their high vitamin C.

Heterobeltiosis is defined as a condition in which the performance of F_1 offspring is superior to the best parent (Karim et al., 2021; Singh et al., 2023). In this study, heterobeltiosis effects were observed in eight traits such as plant height, leaf length, fruit maturity, fruit weight per plant, yield per hectare, ash content, vitamin C and glucose. This finding aligns with Amorim et al. (2021), who reported heterobeltiosis effects in cayenne chili related to fresh fruit weight and dried fruit weight. Compared to *C. annuum*, heterobeltiosis is less frequently reported in *C. frutescens*. Aditika et al. (2020) reported heterobeltiosis in *C. annuum* for plant height, fruit yield per plant, and vitamin C. This pattern suggests a notable potential for heterobeltiosis to contribute to the enhancement of certain traits in *Capsicum* species through selective breeding.

In this study, reciprocal effects were not uniformly observed across all traits between the AB and BA hybrid crosses. The lack of significant differences in the same traits indicates that both male and female parents contribute equally to the traits of their F_1 offspring. However, reciprocal effects were noted on specific eight traits, such as stem length (BA), stem width (AB), fruit weight (BA), ash content (AB), lipid content (AB), flavonoid (BA), fructose (BA) and glucose (BA), with chemical traits like BA exhibiting significantly higher values than AB. Based on the parameters of heterosis, heterobeltiosis, and reciprocal effects, AB and BA are F_1 hybrids that possess advantages over their parents in certain aspects.

The finding corroborates previous research indicating that the yield and quality of hybrid cayenne chili fruits are superior to those of open-pollinated varieties. Particularly noteworthy are the higher level of vitamin C in AB and BA compared to their parent plants A and B. Given that humans cannot synthesize vitamin C, which is crucial for diseases prevention and essential metabolic functions (Smirnoff, 2018). The role of flavonoids is as antioxidants and anti-cancer agents (Sharma et al., 2018), BA hybrids offer significant health benefits.

Additionally, SSR analysis showed that ten SSR markers used could effectively distinguish between the parent plants and the F_1 hybrids, as well as, between the F_1 and reciprocal F_1 hybrids. Such genotyped evaluation is important as it helps account for environmental factors that might influence the phenotypic appearance found in some characters. This is also affirming that the observed difference in F_1 hybrids is genetically based.

CONCLUSIONS

The crossbreeding of two pure lines of hot pepper has successfully produced first-generation (F_1) offspring exhibiting significant heterosis and heterobeltiosis effects, particularly in traits critical to chili fruit production and quality. This study provides valuable insight into these phenomena in hot pepper, a subject that has relatively underexplored. For increasing hot pepper production, the development of superior hot pepper varieties can be directed toward hybrid varieties rather than open-pollinated ones. These hybrids demonstrate the successful amalgamation of dominant genes responsible for improved production and quality. In addition to increasing production, hot pepper hybrids may also support natural medicine supply chains due to their enhanced qualities. Based on the outcomes of this study, the hybrid varieties AB and BA are identified as promising candidates for new superior hybrid chili varieties, potentially leading to increased chili productivity and quality improvements.

Author contribution

Conceptualization: K.M., P.L., D.W.U., N.R.A., R.K., T.K.M., M.S. Data curation of phenotypic/morphology characters: R.K., R.R., P.D., N.R.A., N.G., T.K.M. Data curation of phenotypic/chemical characters: R.K., R.R., N.G., D.M., A.F. Data curation of genotypic characters: K.M., P.L., D.W.U., R.T.T., D.S., K.N. Crop management: R.R., T.K.M. Writing-original draft: R.K., A.F., K.M. Writing-review & editing: R.K., K.M., P.L., D.W.U., M.S., A.F., R.R., R.T.T., D.S., D.M. Supervision and Funding acquisition: K.M. All co-authors reviewed the final version and approved the manuscript before submission.

Acknowledgement

This work was funded by National Research and Innovation Agency, Republic of Indonesia (BRIN).

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