Article

Not peer-reviewed version

Combining Ability of *Capsicum annuum* Hybrid for Antioxi-Dant Activities, Polyphenol Content, α-Glucosidase Inhibitory, Yield, and Yield Components

Muhamad Syukur *, Awang Maharijaya, Waras Nurcholis, Arya Widura Ritonga, Arya Yuda Pangestu, Andi Nadia Nurul Lathifa Hatta, Muhammad Ridha Alfarabi Istiqlal, Abdul Hakim, Zulfikar Damaralam Sahid *

Posted Date: 30 August 2024

doi: 10.20944/preprints202408.2151.v1

Keywords: α-glucosidase inhibition; antioxidant; combining ability; heterotic effect; polyphenol content

Article

Combining Ability of *Capsicum annuum* Hybrid for Antioxi-Dant Activities, Polyphenol Content, α-Glucosidase Inhibitory, Yield, and Yield Components

Muhamad Syukur [1,2,*], Awang Maharijaya [1,2], Waras Nurcholis [3], Arya Widura Ritonga [1,2], Arya Yuda Pangestu [1], Andi Nadia Nurul Lathifa Hatta [4], Muhammad Ridha Alfarabi Istiqlal [5], Abdul Hakim [6] and Zulfikar Damaralam Sahid [1,7,*]

[1] Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University Bogor. Jl. Meranti, IPB Dramaga Campus, Bogor, Indonesia
[2] Center for Tropical Horticulture Studies, IPB University. Jl. Raya Padjajaran, IPB Baranangsiang Campus, Bogor, Indonesia
[3] Department of Biochemistry, Faculty of Mathematics and Natural Sciences, IPB University. Jl. Agatis, IPB Dramaga Campus, Bogor, Indonesia
[4] Research Center for Genetic Engineering, Research Organization for Life Sciences and Environment, National Research and Innovation Agency, Indonesia
[5] Study Program of Agrotechnology, Faculty of Industrial Technology, Gunadarma University. Jl. Margonda Raya, Depok, Indonesia
[6] Study Program of Agrotechnology, Faculty of Agriculture, Siliwangi University. Jl. Peta, Tasikmalaya, Indonesia
[7] Vocational School of Sciences, IPB University. Jl. Kumbang, Bogor, Indonesia
* Correspondence: muhsyukur@apps.ipb.ac.id (M.S.); zulfikards@apps.ipb.ac.id (Z.D.S.); Tel.: +62 822 9900 5933

Abstract: Type II diabetes is a major coronavirus disease comorbidity that can be prevented through a diet. Capsicum annuum (chili) consumption is often suggested, and using functional food cultivars is the most effective strategy. However, the general combining ability (GCA), specific combining ability (SCA), and heterotic effect of functional biochemicals (polyphenol content, antioxidant activities, and α-glucosidase inhibitory compounds) remain poorly known in C. annuum. This study aimed to estimate those parameters in C. annuum by using five different genotypes and their hybrid com-binations based on growth characteristics, yield, yield components, and fruit functional biochem-icals. The F1 and F1R progenies were obtained from crosses in a greenhouse and full diallel design. Each parent used in this study had a GCA advantage for each character. The hybrid combination of 074 x 005 and 435 x 367 displayed the best yield results. However, the results indicated the opposite regarding α-glucosidase inhibitory. The heterotic effect of functional biochemicals was observed for traits related to genotypes, polyphenol content, antioxidant activity, α-glucosidase inhibitory compounds, and similar properties related to yield and yield components, indicating their hybrid use in chili production.

Keywords: α-glucosidase inhibition; antioxidant; combining ability; heterotic effect; polyphenol content

1. Introduction

Chili is generally known as a food ingredient because of its spiciness, whose levels depend on the species [1]. Five common species are cultivated, including *C. annuum* (lowest spiciness) [2] and *C. chinense* (highest spiciness) [3]. Chili spiciness is caused by a secondary metabolite compound called capsaicin and can be quantified using Scoville heat units [4]. Capsaicin is an active component of chili that can irritate and cause a burning sensation in body tissues [5]. Generally, people use chili spiciness in various ways: flavor enhancer [6], self-protection spray [7], cosmetic raw materials [8,9], or for the

 doi:10.20944/preprints202408.2151.v1

pharmaceutical development of chili patches [10–12]. Recent studies reported that C. annuum functional secondary metabolites are more beneficial for human health than other species [13].

The chili plant's most commonly used part is its fruit. The chili fruit has various secondary metabolites with high benefits: capsaicin, alkaloids [14], terpenoids [15], steroids [16], saponins [17], vitamin A [18], vitamin C [18], capsanthin [19], zeaxanthin [20], and cryptoxanthin (used as a dye) [20]. Additionally, chili has various macro minerals (iron, potassium, calcium, phosphorus, and niacin), which are absorbed by the body to maintain human health [21]. In medicine, chili capsaicin is used for its an-algesic properties, relieving asthma and skin itchiness. Additionally, recent studies have reported its anticancer, antidiabetic, and antiarthritis activities, and its role as a gastric acid secretion stimulant to prevent digestive system infections.

Generally, secondary metabolites are produced by plants under environmental stress conditions [22]. However, chili possesses basal antioxidant activity and poly-phenol content [22,23]. Antioxidants counteract free radicals in the body [24], and free radical scavenging helps maintain health and prevent degenerative diseases. One such degenerative disease is diabetes mellitus, which chili can potentially help overcome as a functional food.

The World Health Organization aims to decrease the number of patients with diabetes within the next ten years [25]. Chili consumption can help reach this target through its preventive properties. COVID-19 has changed people's lifestyles world-wide, making them focus primarily on their diet. A healthy lifestyle includes a diet with foods rich in functional biochemical compounds. Consuming functional bio-chemical compounds by eating chili is a precautionary measure and helps reduce the side effects of chemical drugs impacting patients [26]. Our previous hybridization re-search produced a hybrid performance with functional biochemical compounds to help overcome this problem [27]. Additionally, chili has α-glucosidase inhibitory (AGI) compounds, which inhibit blood sugar absorption [27–30].

Chili's functional compounds can be improved in various ways. Plant cultivation techniques can significantly affect the content of active compounds in chili due to en-vironmental influences [31]. One solution is to create new varieties through genetic improvement. New superior varieties can be developed conventionally or with bio-technology methods. Hybridization is a conventional method that produces hybrids with favorable combined properties (yield and biochemical content). The obtained hybrid performance information must be supported by combining ability information as the primary consideration in the selection process. Selecting varieties based on the estimation value of combined abilities for targeted traits will facilitate the development of new chili varieties with high yields and functional biochemical compounds. This study aims to determine the estimated value of combining power in polyphenols, an-tioxidant activity, AGI, yield, and yield components in *C. annuum*.

2. Materials and Methods

2.1. Plant Material

The parent plants used in this study were selected in previous studies [32,33]. Our previous study reported that the IPB breeding laboratory collection genotypes contained polyphenols, antioxidant activity, and α-glucosidase inhibitors. Five genotypes (IPB367, IPB435, IPB005, IPB374, and IPB074) with distinctive values for hybridization activities were selected as parents. IPB367 is an ornamental chili with AGI and high 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant activity. The IPB367 and IPB 435 genotypes have more fruits but with lower weights than other genotypes. Therefore, IPB005, IPB374, and IPB074, which produced a large quantity of chili fruits with high weights, were selected. The hybridization is expected to produce superior progeny regarding two different traits.

Hybrids and reciprocals were produced from hybridization in Bogor, Indonesia, at the IPB Alam Sinarsari greenhouse. The randomized complete block design was used for the full diallel analysis of five parents [27]. Chili was cultivated in a greenhouse under a controlled environment, following chili cultivation procedures.

2.2. Growth, Yield, and Yield Component Evaluation

F1's and F1R's fruits of ten sample plants were evaluated; 20 fruits were harvested from each experimental unit of 25 treatments. The following growth characteristics were measured: dichotomous height (DH) from the base of the soil to the first dichotomous branch (with a digital ruler); leaf length (LL) from base to tip (with a digital ruler); leaf width (LW) from the left end to the right end (with a digital ruler); and stem diameter (SD), at 5 cm high from the ground.

Yield components were also measured: fruit length (FL), using a digital ruler; fruit diameter (FD), calculating the average diameter of ten fruits in one plant in the third to fifth harvests (with a digital caliper); fruit thickness (FT), by calculating the average thickness of ten fruits in one plant in the third to fifth harvests (with a digital caliper); fruit weight (FW), by calculating the average weight of ten fruits in one plant in the third to fifth harvest and weighing it (with a digital analytical balance); the number of fruits per plant (NFP), by counting the total fruit number on one plant during the eight harvests; and yield, by calculating the total fruit weight of one plant during the eight harvests and weighing it (with a digital analytical balance).

2.3. Biochemical Evaluation

Perfectly ripe red chilies were used for biochemical measurements. The following biochemical compounds were sequentially measured: total phenolic content (TPC) with gallic acid standard, total flavonoid content (TFC) with quercetin standard, antioxidant activity of DPPH, ferric reduction antioxidant power method (FRAP) with trolox standard, and AGI with acarbose standard. All measurements were performed by ELISA Reader Spectrophotometry at the following wavelengths: 750, 415, 517, 595, and 410 (following the order cited above). The measured absorbance results were converted using standard charts and analyzed to estimate the combining ability and heterotic effect. The extraction was performed using 3 g of chili powder dissolved in 20 mL of 70% ethanol, and samples were left on an orbital shaker for 48 h. Samples were then filtered and deposited in a vial for 24 h in the refrigerator [34].

2.4. Data Statistical Analysis

General combining ability (GCA), specific combining ability (SCA), and heterotic effect of functional compounds were estimated using the full diallel method following Griffing's methodology [1956; Method I, Model 1 (fixed effect)]. All performance data were analyzed using "Analysis of Genetics Designs" in R (AGD-R) v. 5.0. (Centro Internacional de Mejoramiento de Maíz y Trigo). The raw data were collected from field and laboratory analysis with three replications. Each replication used ten samples for analysis. The statistical model was defined as follows:

$$Yij = m + gi + gj + sij + rij + 1/bc \ \Sigma\Sigma eijkl$$

where *Yij* is the mean value of hybrid and parent genotypes; *m* is the effect of general means; *gi* and *gj* are effects of the GCA related to the *i-th* and *j-th* parent, respectively; *sij* is the effect of SCA for the crossings between the parents of order *i* and *j*; *rij* is the reciprocal effect; and *1/bc ΣΣeijkl* is the mean's value of error.

The critical difference test (CD) was conducted to evaluate differences in GCA between parents. If GCA > CD, the GCA was considered significantly different. The heterotic and heterobeltiosis concerning the mean of the parents was calculated for each character, using the AGD-R program:

$$CD = \sqrt{(MSe/p)} \times t \ (5\% \text{ table})$$

where *CD* is the critical difference test; *MSe* is the mean square error, and *p* is the number of parents.

3. Results

3.1. Analysis of Variance

GCA and SCA estimates were essential components obtained from chili plant breeding activities. Variance analysis in this study significantly affects the GCA mean square for growth characters, yield, yield components, and biochemical content in all observed variables. The SCA mean square also significantly affected all observed variables (Tables 1–3).

Table 1. ANOVA of chili growth characters.

Sources	df	Mean Square			
		DH	LL	LW	SD
GCA	4	219.98**	10.25**	1.71**	6.85**
SCA	10	15.91**	0.95**	0.25**	3.05**
Reciprocal	10	8.91**	1.01**	0.22**	8.35**
Error	48	0.68	0.03	0.01	0.01
Coefficient of Variance (%)		5.03	3.20	4.86	1.61

df, degree of freedom; GCA, general combining ability; SCA, specific combining ability; DH, dichotomous height; LL, leaf length; LW, leaf width; SD, stem diameter; ** significant at $P \leq 0.01$.

Table 2. ANOVA of chili yield and yield components.

Sources	df	Mean Square					
		FL	FD	FT	FW	NFP	Yield
GCA	4	55.76**	85.93**	0.48**	88.41**	39.04**	445617.7**
SCA	10	0.94**	8.05**	0.14**	9.63**	796.63**	35735.5**
Reciprocal	10	0.67**	0.93**	0.06**	0.29**	3.79ns	1008.59**
Error	48	0.03	0.03	0.002	0.05	3.61	307.77
Coefficient of Variance (%)		4.03	2.64	5.03	6.17	4.71	6.81

df, degree of freedom; GCA, general combining ability; SCA, specific combining ability; FL, fruit length; FD, fruit diameter; FT, fruit thickness; FW, fruit weight; NFP, fruit number per plant; ** significant at $P \leq 0.01$; ns, not significant.

Table 3. ANOVA of chili biochemical compounds.

Sources	df	Mean Square				
		TPC	TFC	DPPH	FRAP	AGI
GCA	4	100.18**	0.66**	0.07**	86.75**	500.27**
SCA	10	129.29**	1.31**	0.04**	150.56**	40.73**
Reciprocal	10	1.71**	0.01**	0.002**	3.39**	0.81**
Error	48	0.26	0.002	0.001	0.42	0.38
Coefficient of Variance (%)		3.16	3.20	1.19	3.26	1.60

df, degree of freedom; GCA, general combining ability; SCA, specific combining ability; TPC, total phenolic content; TFC, total flavonoid content; DPPH, antioxidant activities; FRAP, antioxidant activities; AGI, α-glucosidase inhibitor; ** significant at $P \leq 0.01$.

Sources of reciprocal diversity in all variables observed for growth characters, yield, yield components, and biochemical content displayed a significant effect at the 1% level. The significant effect on reciprocity indicated that the F1 and F1 reciprocals tested in this study differed significantly. Significantly different information on the diversity sources in GCA and SCA is the basis for further estimating combining abilities. Thus, the coefficient of variance of diversity in growth characters, yield, and yield components were produced between 1.61%–5.03% and 2.64%–6.81% (Tables 1 and 2). The coefficient of variance of the variable observed for biochemical content was produced between 1.60%–3.26% (Table 3).

3.2. GCA Analysis

IPB005 was the parent genotype with the highest GCA for several growth characters and yield components, including LW, FD, FT, FW, NFP, and yield. The best GCA for SD, LL, and FL characters was displayed by parent IPB374 (Tables 4 and 5). IPB074 had the best GCA for DH and TPC. Meanwhile, IPB435 displayed the best GCA for TFC and FRAP and IPB367 for DPPH and AGI.

Table 4. GCA for chili growth characters.

Genotypes	DH	LL	LW	SD
IPB005	-1.27^{d}	0.56^{b}	0.47^{a}	0.15^{b}
IPB374	3.13^{b}	0.77^{a}	0.32^{b}	1.25^{a}
IPB367	-6.94^{e}	-1.75^{e}	-0.57^{e}	0.06^{b}
IPB435	-0.24^{c}	0.37^{c}	-0.21^{d}	-0.51^{c}
IPB074	5.33^{a}	0.06^{d}	-0.01^{c}	-0.95^{d}
Critical difference	0.79	0.16	0.10	0.10

DH, dichotomous height; LL, leaf length; LW, leaf width; SD, stem diameter; Numbers followed by the same letter in the same column are not significantly different according to the critical difference test at 5%.

Table 5. GCA for chili yield and yield components.

Genotypes	FL	FD	FT	FW	NFP	Yield
IPB005	0.88^{c}	4.47^{a}	0.28^{a}	3.17^{a}	2.14^{a}	239.24^{a}
IPB374	2.57^{a}	-0.39^{c}	0.16^{b}	1.10^{c}	0.87^{a}	69.99^{c}
IPB367	-2.86^{e}	-1.87^{d}	-0.09^{c}	-2.93^{d}	-2.49^{b}	-203.75^{d}
IPB435	-2.11^{d}	-3.14^{e}	-0.27^{d}	-3.38^{e}	1.14^{a}	-238.53^{e}
IPB074	1.53^{b}	0.93^{b}	-0.08^{c}	2.04^{b}	-1.66^{b}	133.05^{b}
Critical difference	0.16	0.16	0.04	0.21	1.81	16.71

FL, fruit length; FD, fruit diameter; FT, fruit thickness; FW, fruit weight; NFP, number of fruits per plant. Numbers followed by the same letter in the same column are not significantly different according to the critical difference test at 5%.

The GCA results for yield and yield components varied between parents (Table 5). Chili lines IPB005 and IPB374 had positive GCA for FL, FT, FW, and yield. Meanwhile, the chili lines IPB367 and IPB435 had negative GCA for the same characters, especially IPB367, which had the highest negative GCA for FL (GCA −2.86) and NFP (GCA −2.49). The IPB435 parent had the highest negative GCA for FD (−3.14), FW (−3.38), and yield (−238.53).

The highest GCA values for five parents were evenly distributed. Each parent displayed advantages in the desired character. The parents IPB005, IPB367, and IPB074 have positive GCA for DPPH antioxidant activities, while IPB374 and IPB435 exhibited the highest negative GCA for DPPH antioxidant activities. Only parents IPB005 and IPB435 had a positive GCA for FRAP antioxidant activities (0.56 and 4.62, respectively). IPB435 had positive GCA results for AGI (3.99) and TFC (0.35). Meanwhile, IPB435 had a negative GCA for TPC (−0.80). IPB367 displayed a positive GCA on all biochemical observations, including TPC, TFC, DPPH, and AGI, except FRAP antioxidant activity, with respective values of 2.18, 0.18, 0.08, 9.86, and −3.07 (Table 6).

Table 6. GCA for chili biochemical contents.

Genotype	TPC	TFC	DPPH	FRAP	AGI
IPB005	-3.08^{d}	-0.14^{c}	0.04^{b}	0.56^{b}	-3.88^{d}
IPB374	-2.59^{d}	-0.26^{d}	-0.09^{c}	-0.22^{c}	-1.65^{c}
IPB367	2.18^{b}	0.18^{b}	0.08^{a}	-3.07^{e}	9.86^{a}
IPB435	-0.80^{c}	0.35^{a}	-0.09^{c}	4.62^{a}	3.99^{b}
IPB074	4.30^{a}	-0.13^{c}	0.05^{ab}	-1.89^{d}	-8.32^{e}
Critical difference	0.49	0.04	0.03	0.62	0.59

 doi:10.20944/preprints202408.2151.v1

TPC, total phenolic content; TFC, total flavonoid content; DPPH, antioxidant activities; FRAP antioxidant activities; AGI, α-glucosidase inhibitor. Numbers followed by the same letter in the same column are not significantly different according to the critical difference test at 5%.

3.3. SCA Analysis

Parents with positive and negative SCA in DH and SD (12 and 11 combinations of crosses) exhibited positive SCA in LL and LW. The highest SCA for DH was displayed by hybrid 374 x 435 (4.33), followed by hybrid 005 x 435 (3.67), 074 x 435 (3.64), and 074 x 374 (3.44). The combined 435 x 374 and 435 x 367 hybrids had a negative SCA for LL (−0.61 and −0.73) and leaf width (−0.47 and −0.37). Positive SCA values for all growth characters were displayed by hybrid 367 x 005, which also had the highest positive SCA values for LL (0.69) (Table 7).

Table 7. SCA for chili growth characters.

Chili Hybrid			DH	LL	LW	SD
005	x	374	-0.33	0.33	0.68	-1.24
005	x	367	1.5	0.4	0.001	-3.62
005	x	435	3.67	0.67	-0.12	0.47
005	x	074	-1.00	0.37	0.4	-1.41
374	x	005	1.37	0.68	0.43	-0.78
374	x	367	-1.83	-0.08	0.001	-3.89
374	x	435	4.33	-1.42	-0.42	2.51
374	x	074	-0.67	-1.18	-0.48	1.64
367	x	005	1.61	0.69	0.13	1.67
367	x	374	-0.46	0.004	0.28	-0.34
367	x	435	-0.17	0.55	0.2	0.62
367	x	074	-1.33	0.13	-0.08	0.59
435	x	005	-2.26	0.61	0.181	-0.69
435	x	374	0.67	-0.61	-0.47	1.37
435	x	367	0.24	-0.73	-0.37	-2.03
435	x	074	-1.83	0.68	0.02	-0.25
074	x	005	-2.16	-0.05	-0.202	0.62
074	x	374	3.44	-0.24	-0.27	2.66
074	x	367	1.17	0.42	0.15	-0.06
074	x	435	3.64	-0.04	-0.02	2.46

DH, dichotomous height; LL, leaf length; LW, leaf width; SD, stem diameter.

Yield and yield components for SCA estimates included FL, FD, FT, FW, and NFP (Table 8). SCA for FL was between −1.12–+1.12. The lowest and highest SCA for FL were in hybrids 005 x 435 and 005 x 374. The three highest SCA values for FD were seen in hybrids 074 x 005, 367 x 005, and 435 x 367 (2.51, 2.49, and 1.74, respectively). Hybrid 074 x 005 had a negative SCA value (-0.43) for FT.

Table 8. SCA for chili yield and yield components.

Chili Hybrids			FL	FD	FT	FW	NFP	Yield
005	x	374	1.12	0.61	-0.26	0.75	0.5	-22.82
005	x	367	0.65	0.15	0.05	0.81	0.83	16.51
005	x	435	-1.12	-0.72	-0.34	-0.04	-0.17	-7.38
005	x	074	-0.28	-0.38	-0.01	-0.19	-3.00	-54.32
374	x	005	0.4	0.66	-0.08	1.78	-5.27	100.69
374	x	367	0.23	-0.67	0.32	-0.37	0.001	-0.31
374	x	435	0.12	0.12	0.003	-0.11	0.5	-1.67
374	x	074	0.03	-1.18	0.11	-0.19	-1.5	-34.32

 doi:10.20944/preprints202408.2151.v1

367	x	005	-0.81	2.49	-0.04	-2.34	8.76	-115.88
367	x	374	-0.38	-0.24	-0.19	-0.76	-18.47	-61.09
367	x	435	0.1	0.28	0.013	0.05	-1.33	2.06
367	x	074	0.001	0.09	-0.04	0.1	-0.17	7.695
435	x	005	-0.56	-2.81	-0.03	-3.07	11.46	-102.67
435	x	374	0.02	1.39	-0.27	-0.79	27.39	17.53
435	x	367	0.46	1.74	0.08	2.77	-28.07	104.03
435	x	074	-0.52	1.28	0.09	-0.09	2.17	-0.61
074	x	005	0.01	2.51	-0.43	2.09	0.76	135.85
074	x	374	-0.99	-0.75	0.26	-1.84	5.19	-90.17
074	x	367	-0.31	-2.13	-0.19	-1.53	16.56	-71.09
074	x	435	0.19	-0.82	0.12	-1.16	-30.74	-206.33

FL, fruit length; FD, fruit diameter; FT, fruit thickness; FW, fruit weight; NFP, number of fruits per plant.

FW, NFP, and FW per plant were the main characteristics that determined potential chili productivity. The highest SCA values for FW were observed in hybrids 074 x 005, 374 x 005, and 005 x 367. The highest SCA for NFP was observed in hybrids 435 x 374, 074 x 367, and 435 x 005. Meanwhile, the highest SCA for FW per plant was for hybrid 074 x 005, which was recommended for high potential yield (Table 8).

Eight hybrids had positive SCA, and 12 had negative SCA for TPC and AGI characters. SCA for antioxidant activity displayed contradictory results depending on the method used. DPPH had 13 positive SCA in contrast to FRAP, which had 13 negative SCA. The hybrid 367 x 374 had the lowest negative SCA for the three biochemical constituents (TPC: −9.57; TFC: −0.89; FRAP: −12.36). The highest SCA for AGI and TPC were observed for hybrid 074 x 374 (7.83 and 4.06). Hybrid 435 x 367 exhibited the highest positive SCA for TFC (1.11) and FRAP (8.23). Finally, hybrid 367 x 374 had the highest positive SCA for DPPH (0.16) (Table 9).

Table 9. SCA for chili biochemical compounds.

Chili Hybrids			TPC	TFC	DPPH	FRAP	AGI
005	x	374	0.17	-0.01	0.001	-0.61	-0.22
005	x	367	-0.28	0.05	-0.03	-0.67	-0.5
005	x	435	-0.42	0.06	0.04	-0.46	0.24
005	x	074	-0.69	-0.03	0.04	0.49	-0.88
374	x	005	-3.26	0.08	-0.1	-3.53	-3.09
374	x	367	-1.01	-0.01	-0.01	0.52	-0.15
374	x	435	0.15	0.14	0.04	3.24	-1.03
374	x	074	-1.12	0.07	0.04	-1.12	-0.2
367	x	005	-6.06	-0.73	0.11	-0.92	0.68
367	x	374	-9.57	-0.89	0.16	-12.36	4.4
367	x	435	-0.35	0.02	0.03	0.88	-0.61
367	x	074	0.52	0.02	0.02	1.39	0.76
435	x	005	-7.39	-0.48	0.07	-6.58	2.99
435	x	374	-7.44	-0.47	-0.014	-1.93	-2.8
435	x	367	7.72	1.11	-0.28	8.23	-0.52
435	x	074	-2.26	-0.03	0.03	-0.97	0.91
074	x	005	2.99	0.76	-0.02	5.96	-6.68
074	x	374	4.06	0.26	0.01	-0.27	7.83
074	x	367	1.65	-0.85	-0.03	-10.17	0.23
074	x	435	0.58	-0.64	0.2	-2.89	-0.004

TPC, total phenolic content; TFC, total flavonoid content; DPPH, antioxidant activities DPPH method; FRAP, antioxidant activities FRAP method; AGI, α-glucosidase inhibitory.

3.4. Heterotic and Heterobeltiosis Effects in Chili

The results of heterotic and heterobeltiosis effect analysis on growth characters, yield components, and biochemical content are presented in Tables 10–12. The heterotic effect for DH was −20.75%–32.95%, whereas the effect of heterobeltiosis was −25.61%–24.24%. The highest heterotic and heterobeltiosis effects for this character were observed in hybrids 435 x 074 and 374 x 074. Hybrid 435 x 374 consistently displayed the second-highest heterotic and heterobeltiosis effects. The highest heterotic effect for leaves (LL and LW) was observed in hybrid 367 x 005; Heterotic and heterobeltiosis effects were 35.98% and 9.36% for LL and 15.46% and −8.94% for LW, respectively. Those effects were the highest for SD in hybrid 435 x 374. The hybrids 367 x 374 and 367 x 435 had the overall lowest heterobeltiosis effect (Table 10).

Table 10. Heterotic and heterobeltiosis effects for chili growth characters.

Chili Hybrids			TD		PD		LD		DBT	
			HMP	HHP	HMP	HHP	HMP	HHP	HMP	HHP
			……………………………………… (%) ………………………………………							
005	x	374	12.28	7.87	13.55	-0.32	0	-3.05	32.79	31.77
005	x	367	3.39	-25.61	23.28	-0.85	15.46	-8.94	68.68	54.86
005	x	435	-20.75	-23.17	5.93	-6.23	5.79	4.07	-3.2	-14.22
005	x	074	3.87	-5.05	7.26	1.92	-12.3	-13.01	30.44	24.99
374	x	005	9.94	5.62	20.88	6.11	32.28	28.24	-2.13	-2.88
374	x	367	24.8	-12.36	2.64	-25.08	10.89	-14.5	59.42	47.39
374	x	435	0.00	-6.74	3.25	2.25	-9.6	-13.74	13.71	0.09
374	x	074	30.85	24.24	9.44	0.64	0.79	-3.05	30.66	24.28
367	x	005	18.64	-14.63	35.98	9.36	15.46	-8.94	-24.63	-30.81
367	x	374	7.2	-24.72	0.44	-26.69	10.89	-14.5	-40.37	-44.87
367	x	435	15.04	-15.58	-19.64	-40.98	-25.26	-40.34	-35.54	-46.96
367	x	074	30.37	-11.11	7.92	-16.48	5.21	-16.53	-18.87	-28.36
435	x	005	6.92	3.66	20.74	6.89	0	-1.63	11.67	-1.04
435	x	374	31.33	22.47	-24.35	-25.08	-29.6	-32.82	93.29	70.14
435	x	367	13.27	-16.88	-4.91	-30.16	-12.63	-30.25	-17.69	-32.27
435	x	074	32.95	18.18	-11.31	-17.7	-13.33	-14.05	48.25	36.6
074	x	005	-2.76	-11.11	16.13	10.34	7.38	6.5	-11.36	-15.07
074	x	374	26.6	20.2	-15.38	-22.19	-22.22	-25.19	78.63	69.91
074	x	367	18.52	-19.19	11.88	-13.41	0	-20.66	-3.18	-14.49
074	x	435	20.45	7.07	3.18	-4.26	-12.5	-13.22	39.97	28.97

DH, dichotomous height; LL, leaf length; LW, leaf width; SD, stem diameter; HMP, Heterotic mid parent (heterotic effect); HHP, Heterotic high parent (heterobeltiosis effect).

Table 11. Heterotic and heterobeltiosis effects for chili yield and yield components.

Chili Hybrids			FL		FD		FT		FW		NFP		Yield	
			HMP	HHP	HMP	HHP	HMP	HHP	HMP	HHP	HMP	HHP	HMP	HHP
			…………………………………………… (%) ……………………………………………											
005	x	374	-13.59	-23.93	-2.94	-30.42	-10.14	-19.01	-4.54	-18.4	10.74	6.91	15.2	-3.06
005	x	367	-35.24	-57.63	-31.14	-48.79	-23.78	-37.53	-60	-76.95	7.16	-10.08	-35.86	-61.46
005	x	435	1.86	-31.78	-23.67	-51.88	-1.53	-29.78	-63.11	-79.4	12.64	-7.97	-33.85	-61.21
005	x	074	-6.57	-12.09	6.46	-12.29	-37.37	-49.27	2.27	-2.71	11.28	-0.89	9	6.85
374	x	005	4.47	-8.03	4.48	-25.1	-31.09	-37.89	8.02	-7.67	12.4	8.51	9.22	-8.09
374	x	367	-18.62	-49.76	-5.74	-10.94	-41.06	-47.06	-35.25	-60.67	-33.18	-42.25	-37.22	-59.34
374	x	435	-5.84	-40.76	19.78	-4.11	-27.49	-44.49	-45.34	-68.19	27.59	7.25	-23.46	-52.04
374	x	074	-15.78	-21.56	2.93	-14.08	-6.04	-16.74	-32.51	-39.73	10.19	1.34	-24.08	-37.13
367	x	005	-16.67	-45.48	-29.39	-47.49	-19.65	-34.14	-39.96	-65.4	9.47	-8.14	-29.8	-57.82

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 August 2024 doi:10.20944/preprints202408.2151.v1

367 x 374	-13.24	-46.45	-18.5	-22.99	-8.48	-17.8	-47.4	-68.05	-33.18	-42.25	-37.37	-59.44
367 x 435	-2.88	-7.34	-4.18	-26.47	-10.23	-25.19	35.16	19.22	-53.18	-54.71	-37.55	-42.01
367 x 074	-17.93	-47.8	-33.84	-42.03	-25.38	-26.52	-51.21	-71.49	2.49	-4.26	-47.32	-68.56
435 x 005	-29.3	-52.65	-33.73	-58.23	-36.16	-54.48	-64.04	-79.93	12.2	-8.33	-36.62	-62.83
435 x 374	-3.2	-39.1	22.69	-1.79	-27.09	-44.19	-49.03	-70.34	28.88	8.33	-24.32	-52.58
435 x 367	2.88	-1.83	2.3	-21.49	-8.41	-23.67	40.48	23.91	-56.18	-57.61	-35.12	-39.76
435 x 074	2.75	-33.24	-28.45	-49.83	-10.42	-24.41	-47.85	-70.56	-56.4	-60.51	-75.44	-85.68
074 x 005	-11.53	-16.76	2.47	-15.58	-37.97	-49.76	-0.61	-5.45	2.26	-8.93	-2.74	-4.66
074 x 374	-15.27	-21.09	-16.15	-30	5.19	-6.79	-35.96	-42.81	5.83	-2.68	-32.86	-44.4
074 x 367	-17.93	-47.8	-32.48	-40.83	-30.38	-31.44	-48.41	-69.85	2.07	-4.65	-44.59	-66.93
074 x 435	-10.36	-41.76	-3.72	-32.48	2.55	-13.48	-50.47	-72.04	-51.2	-55.8	-75.67	-85.81

FL, fruit length; FD, fruit diameter; FT, fruit thickness; FW, fruit weight; NFP, number of fruits per plant; HMP, Heterotic mid parent (heterotic effect); HHP, Heterotic high parent (heterobeltiosis effect).

Table 12. Heterotic and heterobeltiosis effects for chili biochemical compounds.

Chili Hybrids	TPC		TFC		DPPH		FRAP		AGI	
	HMP	HHP	HMP	HHP	HMP	HHP	HMP	HHP	HMP	HHP
	(%)									
005 x 374	-49.24	-51.48	-22.9	-28.41	-2.04	-9.11	-31.27	-38.81	-4.52	-10.41
005 x 367	-42.47	-44.75	-50.94	-60.81	8.38	2.98	-24.68	-27.14	0.71	-9.57
005 x 435	-49.85	-51.7	-31.8	-42.38	3.32	-1.36	-23.39	-28.44	-0.64	-7.29
005 x 074	4.63	-7.49	14.91	12.03	3.1	1.36	-1.92	-5.06	-14.45	-25.27
374 x 005	-48.32	-50.6	-23.68	-29.13	-2.1	-9.17	-33.88	-41.14	-5.24	-11.09
374 x 367	-50.95	-51.19	-60.21	-66.24	10.02	-2.61	-61.68	-64.85	14.69	-2.65
374 x 435	-52.62	-56.31	-42.45	-48.06	-2.25	-5.14	-31.88	-35.33	1.91	-10.35
374 x 074	5.16	-10.58	-20.55	-24.43	4.71	-1.29	-26.33	-36.26	22.68	13.62
367 x 005	-43.96	-46.18	-47.62	-58.16	4.85	-0.37	-27.84	-30.2	-0.64	-10.79
367 x 374	-56.09	-56.31	-60.51	-66.49	8.94	-3.57	-59.53	-62.87	14.24	-3.04
367 x 435	4.65	-3.05	4.73	-2.27	-18.86	-26.21	-3.95	-7.37	3	-1.19
367 x 074	7.96	-7.83	-54.06	-62.58	0.34	-6.18	-55.98	-58.73	3.96	-17.12
435 x 005	-52.3	-54.06	-28.18	-39.32	7.84	2.96	-25.47	-30.38	0.04	-6.65
435 x 374	-51.78	-55.53	-33.41	-39.91	2.11	-0.91	-18.79	-22.89	-1.23	-13.12
435 x 367	2.68	-4.87	5.41	-1.64	-15.93	-23.54	-0.07	-3.62	1.42	-2.7
435 x 074	13.91	4.21	-35.44	-44.28	13.94	10.59	-16.9	-24.69	-0.62	-18.16
074 x 005	0.27	-11.34	12.86	10.03	7.97	6.15	0.54	-2.68	-17.57	-27.99
074 x 374	-1.61	-16.33	-15.03	-19.18	8.99	2.76	-31.3	-40.56	21.92	12.91
074 x 367	11.13	-5.12	-52.66	-61.44	2.34	-4.31	-49.15	-52.33	6.29	-15.26
074 x 435	-1.04	-9.47	-37.46	-46.02	17.73	14.27	-21.47	-28.83	2.34	-15.72

TPC, total phenolic content; TFC, total flavonoid content; DPPH, antioxidant activities DPPH method; FRAP, antioxidant activities FRAP method; AGI, α-glucosidase inhibitor; HMP, Heterotic mid parent (heterotic effect); HHP, Heterotic high parent (heterobeltiosis effect).

The highest effects for fruit length (HMP, 4.47%; HHP, −1.83%) were displayed by two different hybrids, 374 x 005 and 435 x 367. FD and FT resulted in heterotic effect values of −33.84%–22.69% and −41.06%–5.19%. Meanwhile, the heterobeltiosis effect for these two characters was −51.88%–−1.79% and −54.48%–−6.79%, with the highest value displayed by 435 x 374 and 074 x 374. The highest heterotic and heterobeltiosis effects for the three characters (FW, NFP, yield) were observed in 435 x 367, 435 x 374, and 005 x 374. The highest heterobeltiosis values differed in FW character for hybrid 005 x 074, which had the highest value. The heterotic effect for FW per plant was −75.44%–15.20% (Table 11).

The heterotic and heterobeltiosis effects for TPC were −56.09%–13.91% and −56.31%–4.21%, respectively. The highest and lowest effects for this character were displayed by hybrid 435 x 074 and

367 x 374, respectively. The best effects for TFC were observed in hybrid 005 x 074, followed by the reciprocal hybrid 074 x 005. Interestingly, the same highest value was observed with the FRAP method. The best effects for DPPH were seen in hybrid 074 x 435. The heterobeltiosis effect for FRAP was negative for all hybrids. The highest effects for AGI were seen in hybrid 005 x 074, followed by the reciprocal hybrid 074 x 374 (Table 12).

4. Discussion

Improving chili varieties through hybridization remains a common practice among chili breeders. Hybridization takes advantage of Mendel's segregation laws, where genetic carriers of superior traits are exchanged from two parents. The chili breeding milestone begins with improving high productivity and continues with enhancing plant resistance toward pests and diseases. Developing global issues (COVID-19 pose a new challenge for chili breeders to obtain superior varieties as functional foods. This study evaluated five *C. annuum* parents (self-pollinated) and 20 hybrids and their reciprocals regarding the yield and functional biochemical contents, including polyphenols, antioxidants, and AGI compounds.

The performance of hybrids and parents indicates the success of upgrading a particular character. However, at the genetic level, such performance can be predicted through diallel analysis so that plant breeders can select the best parents for further crosses. The diallel analysis can provide information on the estimated effects of the GCA and SCA. This estimation can identify segregated populations with the potential for high genetic variability and superior hybrid combinations [34–37]. Information on GCA and SCA estimation is crucial and is obtained by diallel analysis using the Griffing method [38]. Genetic studies using this method reveal information on the magnitude of the GCA effect, which can indicate the superiority of the dominant parent in additive effects [39]. Additionally, the SCA effect can also be estimated, demonstrating the superiority of dominant hybrids in nonadditive results. This analysis can also indicate the expected behavior of the resulting hybrid population based on the parent GCA [40].

Parents with high GCA values can increase the expression of the resulting hybrids. Meanwhile, a low GCA value indicates that the parent does not differ from the average of the entire diallel. A higher GCA value contributes to increasing the expression of the intended trait. A positive or negative GCA value indicates that the parent is superior or inferior, respectively. However, a low GCA is better for some traits, like disease resistance [41], plant height, and canopy width in ornamental plants [42,43]. A less negative value for yield components refers to decreased potential yield [44]. Information on GCA and SCA is used to determine parents and appropriate breeding methods to improve the selected character in plants [45].

GCA estimates are used to determine parents for future research [46]. A parent with high GCA could also be used for synthetic varieties and hybrid development programs [47]. Variety development based on growth characters can use DH, LL, and short LW. In this study, the IPB367 genotype was the best for chili's ornamental development. In contrast, increased yield and yield components require tall plants, sturdy stems, and broad leaves to maximize photosynthesis and produce large fruits [48].

Variety development programs aiming to increase productivity can rely on parent GCA values (Table 4–6). The highest GCA for yield were observed for IPB005, IPB074, and IPB374. These three genotypes are also recommended to develop new varieties for large fruits. IPB435 had a high GCA for NFP, besides IPB005 and IPB374.

IPB435 had a high combining ability, in contrast to IPB367, for FRAP antioxidants. However, IPB367 had a better GCA than other parents for DPPH and AGI. Therefore, this line is recommended for the pharmaceutical industry, which produces supplements or drugs for diabetes patients. In line with our previous study, these results suggest that parents with high GCA values for capsaicin could be used to develop spicy *C. annuum* varieties [49].

The SCA effect is a reflection produced by the hybrid based on the GCA value. The hybrid 074 x 005 had the highest SCA for yield resulting from the two parents with the highest positive GCA. Interestingly, for the same character in this study, a hybrid combination with the second highest positive value (hybrid 435 x 367) resulted from the two parents with negative GCA values. Additionally, the SCA value of positive yield characters could be produced from two parents with negative GCA in plant height characters [50]. The AGI displayed the same trend with the highest SCA value displayed by 074 x 374, which was produced from two parents with a negative GCA, including one with the highest negative GCA (IPB074). This is caused by positive genes spreading within the parents and covering the negative genes in the partner parents so they can combine well [51]. A high SCA value indicates better or worse performance than expected, depending on the evaluated characteristics. SCA also estimated the effect of nonadditive and epistatic genes [52].

Diallel analysis can also predict heterotic and heterobeltiosis effects. The heterotic effect indicates that the hybrid performance is better than the means of parents, while the heterobeltiosis effect suggests that the hybrid performance is better than the best parents [53–55]. Combinations with the best heterotic and heterobeltiosis effects which poor performance are not easy to use to become hybrid varieties. The highest heterotic effect in leaf (LL and LW) was seen in 367 x 005, which had the highest positive SCA for LL. This result indicates parents with positive and negative GCA produce hybrids with the highest SCA and heterotic effect regarding leaves. A high SCA also results in a high heterotic effect [56]. Meanwhile, heterotic and heterobeltiosis effects for yield were displayed by different combinations (005 x 374 and 005 x 074) with the same female parent (IPB005). This result aligns with our previous research [49] evaluating chili production as a spice with good heterotic effects in the hybrids evaluated.

The highest heterotic and heterobeltiosis effects for TFC, DPPH, and AGI were displayed by 005 x 074, 074 x 114, and 374 x 074. Interestingly, this study's heterobeltiosis effect for antioxidant FRAP was negative for all hybrids. The heterotic effect is formed by crossing two parents with different gene frequencies [57], which explains that the self-pollination genotype has a zero heterotic effect. Several studies have demonstrated the benefits of using chili hybrids by exploiting the heterotic phenomenon [58].

5. Conclusions

IPB005, IPB074, IPB374, IPB367, and IPB435 had high-affinity potential and advantages as parents based on the observed characters. They can form a superior segregated population determined by the direction of the breeding program. The hybrid 374 x 074 is the best combination considering AGI production; it can be developed for the pharmaceutical industry. Additionally, the hybrid 005 x 074 displayed the best yield. All hybrids observed had a heterotic effect; they could be developed for further research. The GCA and SCA values produced in this study can help breeders develop hybrid chili plants. Moreover, the heterosis effect produced in this study confirmed that hybrid means are higher than that of parents.

Author Contributions: Conceptualization, M.S.Y., A.M.A., and W.N.C.; methodology, A.N.N.; software, A.W.R.; validation, M.S.Y., Z.D.S., and O.S.I.; formal analysis, S.M.A; investigation, M.R.A.; writing—original draft preparation, G.R.P., A.Y.P., and A.N.N.; writing—review and editing, A.H.A., and Z.D.S; visualization, A.W.R., A.M.A., and S.M.A.; supervision, W.N.C., M.S.Y., A.M.A.; project administration, Z.D.S. All authors have made equal contributions to this article. All authors have read and agreed to the published version of the manuscripts.

Funding: This research was funded by Indonesian Ministry of Education and Culture through the Applied Research of National Higher Education in 2024 with Muhamad Syukur as the principal investigator.

Acknowledgments: The authors would like to thank the Ministry of Research, Technology and Higher Education of the Republic of Indonesia, Centre of Tropical Horticulture Studies IPB, National Research and Innovation Agency Indonesia and Institut Pertanian Bogor (IPB University).

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Duranova, H.; Valkova, V.; Gabriny, L. Chili peppers (*Capsicum* spp.): The spice not only for cuisine purposes: An update on current knowledge. *Phytochem. Rev.* **2022**, 21, pp. 1379-1413. https://doi.org/10.1007/s11101-021-09789-7.
2. Jo, Y.; Choi, H.; Lee, J.H.; Moh, S.H.; Cho, W.K. Viromes of 15 pepper (*Capsicum annuum* L.) cultivars. *Int. J. Molecular Science.* **2022**, 10507. https://doi.org/10.3390/ijms231810507.
3. Oney-Montalvo, J.E.; Morozova, K.; Ferrentino, G.; Ramirez Sucre, M.O.; Rodríguez Buenfil, I.M.; Scampicchio, M. Effects of local environmental factors on the spiciness of habanero chili peppers (Capsicum chinense Jacq.) by coulometric electronic tongue. *European Food Research and Technology*. **2021**, 247, pp.101-110. https://doi.org/10.1007/s00217-020-03610-z.
4. Lu, M.; Chen, C.; Lan, Y.; Xiao, J.; Li, R.; Huang, J.; Huang, Q.; Cao, Y.; Ho, C.T. Capsaicin – the major bioactive ingredient of chili peppers: bio-efficacy and delivery system. *Food Funct*. **2020**, 11, 2848. https://doi.org/10.1039/d0fo00351d.
5. Xiang, Q; Guo, W; Tang, X; Cui, S; Zhang, F; Liu, X; Zhao, J; Zhang, H; Mao, B; Chen, W. Capsaicin—the spicy ingredient of chili peppers: A review of the gastrointestinal effects and mechanisms. *Trends in Food Science and Technology*. **2021**, 116, pp. 755-765. https://doi.org/10.1016/j.tifs.2021.08.034.
6. Ni, Y; Qian, Y; Jianshe, C; Ian, F. Impact of capsaicin on aroma release and perception from flavoured solutions. *LWT–Food science and technology*. **2021**, 138, 110613. https://doi.org/10.1016/j.lwt.2020.110613.
7. Krishnatreyya, H; Hazarika, H; Saha, A; Chattopadhyay, P. Capsaicin, the primary constituent of pepper sprays and its pharmacological effects on mammalian ocular tissues. *European J. of Pharmacology*. **2021**, 819, pp.114-121. https://doi.org/10.1016/j.ejphar.2017.11.042.
8. Lali´c, M; Soldi´c, A; Lali´c, A; Lali´c, Z; Serti´c, M. Development and validation of an HPLC method for simultaneous determination of capsaicinoids and camphor in over-the-counter medication for topical use. *Molecules*. **2022**, 27, 1261. https://doi.org/10.3390/molecules27041261.
9. Sibel Bayil Oğuzkan. Extraction of capsinoid and its analogs from pepper waste of different genotypes. *Natural Product Communications*. **2019**. https://doi.org/10.1177/1934578X19865673.
10. Łukasz, A; Dagmara, G; Łukasz, M; Paweł, Ł; Jacek, M; Stanisław, W. Properties of capsaicin and its utility in veterinary and human medicine. *Research in Veterinary Science*. **2019**, 123, pp.14-19. https://doi.org/10.1016/j.rvsc.2018.12.002.
11. Issa, A.Y.; ALSalamat, H.A.; Awad, W.B.; Haddaden, R.M.; Aleidi S.M. The impact of pharmaceutical care on the efficacy and safety of transdermal glucosamine sulfate and capsaicin for joint pain. *Int. J. Clin. Pharm*. **2021**, 43, pp.101-106. https://doi.org/10.1007/s11096-020-01113-1.
12. László, S; Bátai, I.Z.; Berkó, S; Csányi, E; Dombi, Á; Pozsgai, G; Bölcskei, K; Botz, L; Wagner, Ö; Pintér, E. Development of capsaicin-containing analgesic silicone-based transdermal patches. *Pharmaceuticals*. **2022**. https://doi.org/10.3390/ph15101279.
13. Sharma, D; Shree, B; Kumar, S; Kumar, V; Sharma, S. Stress induced production of plant secondary metabolites in vegetables: Functional approach for designing next generation super foods. *Plant Physiology and Biochemistry*. **2022**, 192, pp.252-272. https://doi.org/10.1016/j.plaphy.2022.09.034.
14. Zhang, D; Sun, X; Battino, M; Wei, X; Shi, J; Zhao, L; Liu, S; Xiao, J; Shi, B; Zou, X. A comparative overview on chili pepper (Capsicum genus) and sichuan pepper (Zanthoxylum genus): From pungent spices to pharma-foods. *Trends in Food Science and Technology*. **2021**, 117, pp.148-162. https://doi.org/10.1016/j.tifs.2021.03.004.
15. Hernández, F.C.; González, P.A.; Martínez, O.; Ortiz, J.J.O. Placenta, pericarp, and seeds of tabasco chili pepper fruits show a contrasting diversity of bioactive metabolites. *Metabolites*. **2019**. https://doi.org/10.3390/metabo9100206
16. Fabio, E.; Rosario, R.; Fredy, Q.; Guillaume, C.; Guillaume, M. Metabolomic characterization of 5 native Peruvian chili peppers (Capsicum spp.) as a tool for species discrimination. *Food Chemistry*. **2022**. https://doi.org/10.1016/j.foodchem.2022.132704
17. Francesco, D.G. and Spyridon, A. Petropoulos, phytoestrogens, phytosteroids and saponins in vegetables: Biosynthesis, functions, health effects and practical applications. *Advances in Food and Nutrition Research: Academic Press*. **2019** https://doi.org/10.1016/bs.afnr.2019.02.004
18. Azlan, A.; Sultana, S.; Huei, C.S.; Razman, M.R. Antioxidant, anti-obesity, nutritional and other beneficial effects of different chili pepper: A Review. *Molecules*. **2022**. https://doi.org/10.3390/molecules27030898

19. Xu, J.; Lin, J.; Peng, S.; Zhao, H.; Wang, Y.; Rao, L.; Liao, X.; Zhao, L. Development of an HPLC-PDA method for the determination of capsanthin, zeaxanthin, lutein, β-cryptoxanthin and β-carotene simultaneously in chili peppers and products. *Molecules*. **2023**. https://doi.org/10.3390/molecules28052362
20. Villa-Rivera, M.G. and Ochoa-Alejo, N. Chili pepper carotenoids: Nutraceutical properties and mechanisms of action. *Molecules*. **2020**. https://doi.org/10.3390/molecules25235573
21. Bhatti, S.; Baig, J.A.; Kazi, T.G. Macro and micro mineral composition of Pakistani common spices: A case study. *Food Measure*. **2019**. https://doi.org/10.1007/s11694-019-00173-w
22. Jan, R.; Asaf, S.; Numan, M.; Lubna; Kim, K.M. Plant secondary metabolite biosynthesis and transcriptional regulation in response to biotic and abiotic stress conditions. *Agronomy*. **2021**. https://doi.org/10.3390/agronomy11050968
23. Alonso-Villegas, R.; González-Amaro, R.M.; Figueroa-Hernández, C.Y.; Rodríguez-Buenfil, I.M. The genus *Capsicum*: A review of bioactive properties of its polyphenolic and capsaicinoid composition. *Molecules*. **2023**. https://doi.org/10.3390/molecules28104239
24. Adwas, A.A.; Elsayed, A.S.I.; Azab, A.E.; Quwaydir, F.A. Oxidative stress and antioxidant mechanisms in human body. *J. Appl. Biotech. Bioeng*. **2019**, 6, pp. 43-47. https://doi.org/10.15406/jabb.2019.06.00173
25. Cheng, A.Y.Y.; Gomes, M.B.; Kalra, S.; et. al. Applying the WHO global targets for diabetes mellitus. *Nat. Rev. Endocrinol*. **2023**, 19, pp. 194-200. https://doi.org/10.1038/s41574-022-00793-1
26. Stout, A.; Friedly, J.; Standaert, C.J. Systemic absorption and side effects of locally injected glucocorticoids. *PM&R: Journal of Injury, Function and Rehabilitation*. **2019**, 11, pp. 409-419. https://doi.org/10.1002/pmrj.12042
27. Syukur, M.; Maharijaya, A.; Nurcholis, W.; Ritonga, A.W.; Istiqlal, M.R.A.; Hakim, A.; Sulassih, S.; Perdani, A.Y.; Pangestu, A.Y.; Hatta, A.N.N.L.; et al. Biochemical and Yield Component of Hybrid Chili (Capsicum annuum L.) Resulting from Full Diallel Crosses. Horticulturae 2023, 9, 620. https://doi.org/10.3390/horticulturae9060620
28. Delgado-Velandia, M.; Gonzalez-Marrachelli, V.; Domingo-Relloso, A.; et al. Healthy lifestyle, metabolomics and incident type 2 diabetes in a population-based cohort from Spain. *Int. J. Behav. Nutr. Phys. Act*. **2022**. https://doi.org/10.1186/s12966-021-01219-3
29. Si, M.; Wenxuan, Z.; Xiangnan, Z.; Yuhang, W.; Tong, L.; Xianghong, W. Enhanced hypoglycemic bioactivity via RAS/Raf-1/MEK/ERK signaling pathway by combining capsaicin and quercetin from chili peppers. *Mol. Nutr. Food Res*. **2023** https://doi.org/10.1002/mnfr.202200577
30. Meiqi, L.; Xi, B.; Xueting, Z.; Hongbing, R.; Shengbao, C.; Xiaosong, H.; Junjie, Y. Exploring the phytochemicals and inhibitory effects against α-glucosidase and dipeptidyl peptidase-IV in Chinese pickled chili pepper: Insights into mechanisms by molecular docking analysis. *LWT*. **2022**. https://doi.org/10.1016/j.lwt.2022.113467.
31. Khaitov, B.; Yun, H.J.; Lee, Y.; Ruziev, F.; Le, T.H.; Umurzokov, M.; Bo, B.A.; Cho, K.M.; Park, K.W. Impact of organic manure on growth, nutrient content and yield of chilli pepper under various temperature environments. *Int. J. Env. Res. Pub. Health*. **2019**. https://doi.org/10.3390/ijerph16173031
32. Sahid, Z.D.; Syukur, M.; Maharijaya, A.; Nurcholis, W. Total phenolic and flavonoid contents, antioxidant, and α-glucosidase inhibitory activities of several big chili (*Capsicum annuum* L.) genotypes. *Ciência Rural*. **2022**. https://doi.org/10.1590/0103-8478cr20210913
33. Sahid, Z.D.; Syukur, M.; Maharijaya, A.; Nurcholis, W. Total phenolics, flavonoids, antioxidant activity, and α-glucosidase inhibitory activity of ornamental pepper and several other lines. *Ornamental Horticulture*. **2022**, 28, pp. 230-238. https://doi.org/10.1590/2447-536X.v28i2.2424
34. Sahid, Z.D.; Syukur, M.; Maharijaya, A.; Nurcholis, W. Polyphenol content and pharmacological activities of *Capsicum frutescens* and *C. chinense* genotypes. *Biodiversitas Journal of Biological Diversity*. **2021**. https://doi.org/10.13057/biodiv/d220929
35. Gramaje, L.V.; Caguiat, J.D.; Enriquez, J.O.S.; dela Cruz, Q.D.; Millas, R.A.; Carampatana, J.E.; Tabanao, D.A.A. Heterotic and combining ability analysis in CMS hybrid rice. *Euphytica*. **2020**, 216, pp. 1-22. https://doi.org/10.1007/s10681-019-2542-y
36. Amorim, A.F.S.; Gilio, T.A.S.; de Jesus, J.B.; de Souza, L.H.A.; dos Anjos, I.V.; Araujo, K.L.; Neves, L.G. Genetic improvement of *Capsicum frutescens*: hybrid vigor for anthracnosis resistance and production traits. *Euphytica*. **2021**, 217, pp. 72. https://doi.org/10.1007/s10681-021-02810-2
37. Sran, T.S. and Jindal, S.K. Assessment of heterotic and combining ability effects along with genotype×environment factors influencing the variation of yield and quality components in pepper. *Scientia Horticulturae*. **2022**. https://doi.org/10.1016/j.scienta.2022.111040

38. Mohammadi, M.; Mirlohi, A.; Majidi, M.M.; Soleimani, K.E. Emmer wheat as a source for trait improvement in durum wheat: A study of general and specific combining ability. *Euphytica*. **2021**, 217, pp. 1-20. https://doi.org/10.1007/s10681-021-02796-x
39. Walkowiak, M.; Spasibionek, S.; Krótka, K. Variation and genetic analysis of fatty acid composition in flax (Linum usitatissimum L.). *Euphytica*. **2022**. https://doi.org/10.1007/s10681-021-02941-6
40. Hernández-Mendoza, F.; Torres, T.C.; Rincón, V.H.A.; Gaytán, V.G.; Merino, F.C.G. Genetic components of characters related to yield in pepper guajillo: Method II of Griffing. *Emirates Journal of Food and Agriculture*. **2021**. https://doi.org/10.9755/ejfa.2021.v33.i2.2568
41. Zongo, A.; Konate, A.K.; Koïta, K.; Sawadogo, M.; Sankara, P.; Ntare, B.R.; Desmae, H. Diallel analysis of early leaf spot (*Cercospora arachidicola* Hori) disease resistance in groundnut. *Agronomy*. **2019**. https://doi.org/10.3390/agronomy9010015
42. Pessoa, A.; Rego, E.R.; Santos, C.A.D.; Carvalho, M.G.; Mesquita, J.C.D.; Rego, M.M. Potential of pepper plant accessions for ornamental purposes using diallel analysis. *Anais da Academia Brasileira de Ciências*. **2019**. https://doi.org/10.1590/0001-3765201920180379
43. Marcelino, R.A.G. and Albuquerque, A.S. Diallel analysis of quantitative characteristics in ornamental peppers. *Genetics and Molecular Research*. **2019**
44. Javed, A.; Nawab, N.N.; Gohar, S.; Akram, A.; Javed, K.; Sarwar, M.; Mallhi, A.R. Genetic analysis and heterotic studies in tomato (*Solanum lycopersicum* L.) hybrids for fruit yield and its related traits. *SABRAO J. Breed. Genet.* **2022**, 54, 3, pp. 492-501.
45. Abed, H.W. and Hassan, H.B. Study the GCA and SCA effects of five inbred lines of maize according to half diallel mating system. *Al-Qadisiyah J. Agriculture Sciences*. **2020**, 10, 2, pp. 343-348.
46. Azad, A.K.; Sarker, U.; Ercisli, S.; Assouguem, A.; Ullah, R.; Almeer, R.; Peluso, I. Evaluation of combining ability and Heterotic of popular restorer and male sterile lines for the development of superior rice hybrids. *Agronomy*. **2022**. https://doi.org/10.3390/agronomy12040965
47. Oliveira, G.H.F.; Revolti, L.T.M.; Buzinaro, R.; Charnai, K.; Giorgenon, C.H.B.; Môro, G.V. Combining ability and analysis of genetic components of synthetic maize populations using a mixed model approach. *Revista Brasileira de Ciências Agrárias*. **2019**. https://doi.org/10.5039/agraria.v14i1a5626
48. Qaim, M. Role of new plant breeding technologies for food security and sustainable agricultural development. *Applied Economic Perspectives and Policy*. **2020**, 42, 2, pp. 129-150. https://doi.org/10.1002/aepp.13044
49. Sahid, Z.D.; Syukur, M.; Maharijaya, A. Combining ability and heterotic effects of chili pepper (*Capsicum annuum* L.) genotypes for yield components and capsaicin content. *SABRAO J. Breeding and Genetics*. **2020**, 52, 4, pp. 390-401.
50. Jawarkar, A.K.; Kale, V.S.; Nagre, P.K.; Sonkamble, A.M.; Jadhav, P.V.; Dikey, H.H. Combining ability analysis in chilli hybrids. *Pharma Innovation Journal*. **2023**, 12, 2, pp. 2311-2314.
51. Al-Mamun, M.; Rafii, M.Y.; Misran, A.B.; Berahim, Z.; Ahmad, Z.; Khan, M.M.H.; Oladosu, Y. Combining ability and gene action for yield improvement in kenaf (*Hibiscus cannabinus* L.) under tropical conditions through diallel mating design. *Scientific Reports*. **2022**. https://doi.org/10.1038/s41598-022-13529-y
52. Viana, J.M.S. The impact of epistasis in the heterosis and combining ability analyses. *Frontiers in Plant Science*. **2023**. https://doi.org/10.3389/fpls.2023.1168419
53. Labroo, M.R.; Studer, A.J.; Rutkoski, J.E. Heterotic and hybrid crop breeding: a multidisciplinary review. *Frontiers in Genetics*. **2021**. https://doi.org/10.3389/fgene.2021.643761
54. Schwarzwälder, L.; Thorwarth, P.; Zhao, Y.; Reif, J.C.; Longin, C.F.H. Hybrid wheat: quantitative genetic parameters and heterotic for quality and rheological traits as well as baking volume. *Theoretical and Applied Genetics*. **2022**, 135, 4, pp. 1131-1141. https://doi.org/10.1007/s00122-022-04039-6
55. Wolko, J.; Dobrzycka, A.; Bocianowski, J.; Bartkowiak-Broda, I. Estimation of heterotic for yield-related traits for single cross and three-way cross hybrids of oilseed rape (*Brassica napus* L.). *Euphytica*. **2019**. https://doi.org/10.1007/s10681-019-2482-6
56. Khamphasan, P.; Lomthaisong, K.; Harakotr, B.; Scott, M.P.; Lertrat, K.; Suriharn, B. Combining ability and heterotic for agronomic traits, husk and cob pigment concentration of maize. *Agriculture*. **2020**. https://doi.org/10.3390/agriculture10110510

doi:10.20944/preprints202408.2151.v1

57. Viana, J.M.S.; Risso, L.A.; Oliveira deLima, R.; Fonseca e Silva, F. Factors affecting heterotic grouping with cross-pollinating crops. *Agronomy Journal*. **2021**, 113, 1, pp. 210-223. https://doi.org/10.1002/agj2.20485
58. Karim, K.M.; Rafii, M.Y.; Misran, A.B.; Ismail, M.F.B.; Harun, A.R.; Khan, M.M.H.; Nazneen, F. Current and prospective strategies in the varietal improvement of chilli (*Capsicum annuum* L.) specially heterotic breeding. *Agronomy*. **2021**. https://doi.org/10.3390/agronomy11112217