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Combining Ability on Yields, Capsinoids and Capsaicinoids in Pepper Varieties (*Capsicum annuum* L.)

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Abstract: The requirement for good parental lines of pepper (*Capsicum annuum* L.) with high phytochemicals, especially for capsinoids (CATs) and capsaicinoids (CAPs), is rapidly increasing, and plant breeders are encouraged to develop new pepper varieties. The objective of this study was to estimate the general combining ability (GCA) and specific combining ability (SCA) for the contents of CATs and CAPs in pepper in two different environments. The mean performances for fresh yield, dry yield and phytochemical contents in *C. annuum* L. were significantly affected by the environment. The effect of additive gene action was significant in determining the traits of CAT, Sum CATs, CAT yield and Sum CAPs. Conversely, non-additive gene action played a crucial role in the accumulation of DI-CAT in this population. The parental lines 203, 201, 101 and 202 were identified as the best parents for fruit yield, sum CAPs, sum CATs and CAT yield, respectively, based on their high positive GCA values and mean actual values. The SCA estimates for fruit yield, sum CAPs and sum CATs were positive and high for the hybrids 102/203, 101/201 and 102/202, indicating that they hold promise for use in commercial hybrids.

Keywords: Chili; capsiate; capsaicin; F_1 hybrids; gene action; breeding

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1. Introduction

The development of pepper varieties with high capsiate (CAT) is important for CAT production, particularly for use in health food and pharmaceutical products [1]. The information on genetic resource variation in CAT is required, especially for non-pungent pepper varieties. Currently, high capsiate varieties are more popular due to their health benefits [2]. Non-pungent pepper is more palatable than pungent pepper because of the lack of burning sensation. CAT has an advantage over CAP in terms of biomedical uses, and the demand for CAT is continuously increasing in the pharmaceutical industry [3]. Furthermore, the information regarding gene effects and the best parental lines for CAT content is considerably limited. The information regarding the genetic relationship of the parental lines is one of the most important criteria for breeding programs [4]. The information regarding combining ability is important for the selection of the best parental lines, and the information regarding gene effects controlling quantitative traits is a essential for designing selection programs [5].

General combining ability (GCA) and specific combining ability (SCA) are used to estimate breeding value in plants [6]. According to Barnard et al. [7] and Pandey et al. [8], the parents with high and positive GCA effects for CAT (specifying the trait) could generate hybrids with high and positive SCA effects. As CAP is an economically important phytochemical in *Capsicum* spp., combining ability analysis is commonly used to identify the best parents for CAP in various capsicum populations. In pungent pepper, the GCA and SCA effects were significant for capsaicinoids (CAPs) in *Capsicum pubescens*, indicating that the genes with additive and non-additive effects control the inheritance of this trait [9]. However, the number of genes controlling the inheritance of CAPs is still undetermined. Polygenic inheritance has been reported in [10]. CAPs accumulation is affected by environmental conditions and genetic constitutions. In contrast to CAPs, the information of combining ability for CAT is limited. The gene effects of CAT in pepper have not been clearly investigated. A few studies have reported that the biosynthesis of capsinoids (CATs) is controlled by a single recessive gene [11,12]. However, a single dominant gene controlling CATs synthesis has been reported in different capsicum populations [13,14]. Hence, polygenic genes might be determined based on the continuous distribution of CATs in different pepper populations. To improve the new pepper varieties with high values of fruit yield, CAPs and CATs, further information regarding the combining ability for these traits is necessary.

In this study, six pepper varieties (*C. annuum* L.) with different levels of the sum of capsiate and dihydrocapsiate (sum CATs) and the sum of capsaicin and dihydrocapsaicin (sum CAPs) were selected from our previous work. The aim of this study was to estimate the general combining ability (GCA) and specific combining ability (SCA) for the contents of CATs and CAPs in pepper to identify the good parents. The information obtained in this study may provide a better understanding of the inheritance of CATs and CAPs and help plant breeders to design the appropriate breeding strategies in breeding programs.

2. Materials and Methods

2.1. Plant Materials and Experimental Design

The pepper varieties (*C. annuum* L.) with different levels of sum CATs and sum CAPs selected from previous studies [15] were used as parents in this study. The parents were matched using the North Carolina Design II mating plan in order to produce nine experimental hybrids. This step was taken because there were two different groups of parents. Meanwhile, the North Carolina Design II was employed for the assessment of inbred lines in terms of their general combining ability and particular combining ability, as well as for the estimation of genetic variations.

Six parents, nine experimental hybrids and a check (Table 1) were evaluated using a randomized complete block design with three replications in two environments (Figure 1), namely Kyoto University (KU) (latitude 35° 01′ N, longitude 135° 46′ E, 50-m MASL), Kyoto, Japan, from April to September 2015 under a greenhouse and Khon Kaen University (KKU) (latitude 16° 28′ N, longitude 102° 48′ E, 200-m MASL), Khon Kaen province, Thailand, from April to September 2016 under a plastic-net house. The plot size consisted of five plants. The parents and the check were not used during combining ability analysis, though they were used for mean comparison.

Table 1. Six parental lines used in this study.

Varieties		Pedigree Name	Sum CATs and Sum CAPs Levels	Source
Female parents	101	509-45-16-1-1-1	High Sum CATs	USDA, USA
	102	Jindanil 80	Medium Sum CATs	KKU, Thailand
	103	YTP18-1-10-13-1-1	Low Sum CATs	KKU, Thailand
Male parents	201	Perennial	High Sum CAPs	USDA, USA
	202	Yodsonkhem 80	Medium Sum CAPs	KKU, Thailand
	203	Yuyi 80	Low Sum CAPs	KKU, Thailand
Commercial cultivar check	301	Super-hot	Medium Sum CAPs	East-West Seed

USDA: The United States Department of Agriculture: KKU: Khon Kaen University.

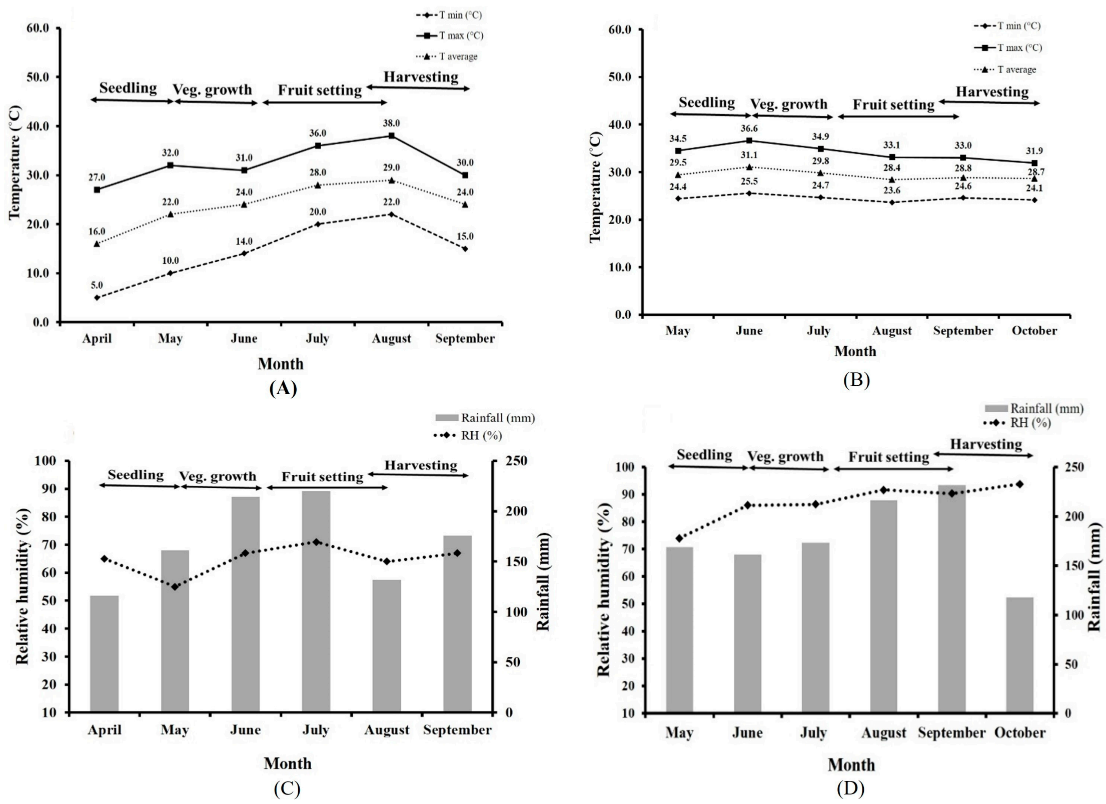

Figure 1. Air temperature at Kyoto University, Japan, from April to September 2015 (**A**); air temperature at Khon Kaen University, Thailand, from May to October 2016 (**B**); relative humidity at Kyoto University (**C**); and relative humidity at Khon Kaen University (**D**).

2.2. Determination of Capsinoids and Capsaicinoids

Twenty green mature fruits per plant were harvested 30 days after anthesis (DAA), and twenty ripe mature fruits per plant harvested at 40 DAA were used to perform the analysis of CATs and CAPs. The fruits were rinsed and dried completely in freeze dryers (Scanvac coolsafe55-9 Model, LaboGene, Denmark) at −53 °C for 65 h. Dried fruits were ground in a blender at room temperature. The ground samples were used to perform the determination of CATs according to the method described by Tanaka et al. [16]. A 4-milliliter volume of acetone (Sigma-Aldrich, St. Louis, MO, USA) was added to 0.2 mg of the dry ground sample. After 1 min of vortexing, the sample tube was allowed to settle for 15 min at room temperature. The supernatant was collected, and 1 mL of acetone was added to the residue. The supernatant was again collected. After repeating this process, 1 mL of ethyl acetate (Sigma-Aldrich, St. Louis, MO, USA) was added to the residue, and the supernatant was collected. The combined supernatant volume was completely evaporated using a rotary evaporator (Speed Vac, LaboGene, Denmark) at 36 °C under vacuum. The residue was dissolved in 2 mL of ethyl acetate, and this solution was filtered into a 2-milliliter glass vial using a Sep-Pak Cartridge C18 (Waters, Milford, MA, USA) and used to perform HPLC analysis. The separation was performed on a μ-Bondapak C18 column (5 μm, 4.6 mm × 250 mm, Inertsil, GL Sciences Inc., Tokyo, Japan) coupled with a guard column (μ-Bondapak Guard-Pak, Waters, Milford, MA, USA). The absorbance of the sample was detected at 280 nm via a UV detector. The solvent was a mixture of MeOH and H_2O (80:20 v/v), with a flow rate of 1.0 mL/min used to perform HPLC analysis (Shimadzu-Model, 10AT-VP series, Kyoto, Japan). Sum CAT and sum CAP contents were calculated as the sum of capsiate (CAT) and dihydrocapsiate (DI-CAT) and capsaicin (CAP) and dihydrocapsaicin (DI-CAP), respectively. Sum CATs and sum CAPs were expressed as μg per g of dry weight (μg/g DW). *CAT yield* was calculated by multiplying CAT content by fruit dry weight using the following formula:

$$CAT\ Yield = \frac{[\text{capsiate (mg)} \times \text{ fruit dry weight}]}{[\text{sample weight}]} \quad (1)$$

2.3. Statistical Analysis

Analysis of variance was performed separately for all entries (parents, hybrids and check), as well as the F_1 hybrids, according to a randomized complete block design. Means were separated using Duncan's multiple range test at a 0.05 probability level [17]. Two-environment data of hybrids were combined to estimate the general and specific combining abilities, but data derived from individual environments were analyzed for all entries because of the heterogeneity of error variances.

Variation among hybrids was partitioned into variation due to male parent, female parent, and the interaction between male and female parents. The mean squares for male parents and female parents are independent estimates of male GCA and female GCA effects, respectively [18]. The male–female interaction mean square is an estimate of the SCA effect. The statistical model used to combine ability analysis followed the method of Singh and Chaudhary. [19]

3. Results

3.1. Combining Ability Analysis

The environments were significantly different ($p \leq 0.01$) for all traits (Table 2). Environment contributed to the large portions of total variations in fresh (27.7%) and dry yields (18.7%), but it contributed to small portions of total variations in sum CAPs (0.0%), CAT (1.6%), DI-CAT (3.3%), sum CATs (1.8%) and CAT yield (4.6%).

Hybrids were also significantly different for all traits. Variations among hybrids were then partitioned into variations due male GCA, female GCA, SCA, male GCA × E, female GCA × E and SCA × E, and these sources of variations were significant ($p \leq 0.05$ and 0.01) for most traits, except for male GCA × E for fresh and dry yield.

Male GCA contributed to the largest portion (91.2%) of total variation in sum CAPs, whereas female GCA contributed to the largest portions of total variations in CAT (59.1%), sum CATs (59.8%) and CAT yield (57.8%). The largest proportions of total variations in fresh yield (34.8%), dry yield (59.0%) and DI-CAT (53.7%) were caused by SCA.The total contributions of GCA (sum of GCA male and GCA female) accounted for more than 80.0% SS for sum CAPs, CAT, sum CATs and CAT yield, and the GCA effects were greater than the SCA effects. However, SCA effects were greater than GCA effects for fresh yield, dry yield and DI-CAT, and the SCA effects accounted for 67.5, 87.1 and 61.0%, respectively. The contributions of male GCA × E, female GCA × E and SCA × E, although they were significant for most traits, were generally low (not larger than 10%) compared to SCA effects and GCA effects, except for female GCA × E for CAT (18.8%) and SCA × E for fresh yield (15.0%). Although differences between environments were significant ($p \leq 0.01$) for all characters, the highest differences were observed for fresh and dry yields, whereas the differences between environments for phytochemical parameters were rather small compared to genotypic differences (Table 3). KKU had higher values than KU for all characters.

Table 2. North Carolina II analysis for fruit yield, sum CAPs, CAT, DI-CAT, sum CATs and CAT yield of the nine tested single crosses derived from 3 × 3 factorial cross combinations assessed across two environments (Kyoto University (KU), Japan, during the spring of 2015 and Khon Kaen University (KKU), Thailand, during the rainy season of 2016).

Source of Variation	*df*	Mean Squares [Percentage of Sum Squares]						
		Fresh Yield (g/plant)	Dry Yield (g/plant)	Sum CAPs (µg/g DW)	CAT (µg/g DW)	DI-CAT (µg/g DW)	Sum CATs (µg/g DW)	CAT Yield (mg/plant)
Replication	2	1000.0	114.2	3433.9	1.6	0.01	1.6	12.8
		[0.5]	[0.4]	[0.0]	[0.0]	[0.0]	[0.0]	[0.0]
Environment (E)	1	120,362.0 **	11,016.2 **	161,801.0 **	2511.9 **	43.6 **	3217.6 **	5558.9 **
		[27.7]	[18.7]	[0.0]	[1.6]	[3.3]	[1.8]	[4.6]
GCA Male	2	26,538.0 **	1206.6 **	191,100,000.0 **	14,583.2 **	53.7 **	16,342.4 **	11,960.9 **
		[12.2]	[4.1]	[91.2]	[18.5]	[8.0]	[18.7]	[19.6]
GCA Female	2	9908.0 **	1373.0 **	8,564,656.0 **	46,575.8 **	176.6 **	52,127.0 **	35,248.9 **
		[4.6]	[4.7]	[4.1]	[59.1]	[26.3]	[59.8]	[57.8]
SCA	4	37,828.0 **	8683.3 **	2,744,214.0 **	7417.2 **	180.3 **	7545.0 **	4226.9 **
		[34.8]	[59.0]	[2.6]	[0.4]	[53.7]	[17.3]	[13.9]
GCA Male × E	2	768.0 ns	80.4 ns	1,903,591.0 **	291.0 **	13.6 **	424.6 **	340.5 **
		[0.4]	[0.3]	[0.9]	[0.7]	[2.0]	[0.5]	[0.6]
GCA Female × E	2	3564.0 **	616.6 **	273,700.0 **	552.5 **	16.2 **	722.6 **	998.4 **
		[1.6]	[2.1]	[0.1]	[18.8]	[2.4]	[0.8]	[1.6]
SCA × E	4	16,319.0 **	1258.6 **	949,474.0 **	99.4 *	12.2 **	145.9 **	319.9 **
		[15.0]	[8.5]	[0.9]	[0.3]	[3.6]	[0.3]	[1.1]
Pooled Error	34	419.0	38.9	7927.1	30.9	0.2	34.8	29.6
		[3.3]	[2.2]	[0.1]	[0.7]	[0.6]	[0.7]	[0.8]
%SS GCA Male		23.7	6.0	93.2	23.7	9.1	19.6	21.5
%SS GCA Female		8.8	6.9	4.2	75.8	29.9	62.4	63.3
%SS SCA		67.5	87.1	2.7	0.5	61.0	18.1	15.2
CV (%)		4.0	3.7	2.1	9.4	10.4	9.2	10.5

df = degree of freedom; CV = coefficient of variation; GCA = general combining ability; SCA = specific combining ability; [%SS] = proportional contribution of sum square. ** and * are significant at $p \leq 0.01$ and $p \leq 0.05$, respectively; ns = not significant.

Table 3. Means for fruit yield, sum CAPs, CAT, DI-CAT, sum CATs and CAT yield of six parents and nine crosses at Kyoto University (KU), Japan, during the spring of 2015 and Khon Kaen University (KKU), Thailand, during the rainy season of 2016.

Varieties	Fresh Yield (g/plant)		Dry Yield (g/plant)		Sum CAPs (μg/plant)		CAT (μg/g DW)		DI-CAT (μg/g DW)		Sum CATs (μg/g DW)		CAT Yield (mg/plant)	
	KU	KKU	KU	KKU	KU	KKU	KU	KKU	KU	KKU	KU	KKU	KU	KKU
101	348.9 ^{g}	519.0 ^{df}	116.3 ^{f}	173.0 ^{df}	0.0 ^{h}	0.0 ^{l}	731.4 ^{a}	924.9 ^{a}	508.4 ^{a}	627.3 ^{a}	1240.0 ^{a}	1552.2 ^{a}	720.9 ^{a}	1344.7 ^{a}
102	468.9 ^{d}	483.0 ^{fg}	156.3 ^{d}	161.1 ^{fg}	1777.9 ^{g}	2519.6 ^{h}	82.6 ^{cd}	151.7 ^{b}	74.3 ^{b}	98.3 ^{b}	156.9 ^{b}	250.0 ^{b}	123.2 ^{b}	201.5 ^{b}
103	344.2 ^{g}	551.0 ^{be}	114.8 ^{f}	183.6 ^{bd}	1919.6 ^{g}	2358.5 ^{h}	48.8 ^{e}	60.7 ^{d}	45.9 ^{c}	6.3 ^{e}	94.7 ^{e}	67.0 ^{ef}	54.3 ^{cd}	61.5 ^{dg}
201	306.2 ^{h}	507.0 ^{ef}	102.1 ^{g}	168.9 ^{ef}	6616.3 ^{b}	7322.4 ^{b}	12.6 ^{gi}	16.0 ^{ef}	11.9 ^{e}	14.0 ^{de}	24.5 ^{g}	29.9 ^{gh}	12.5 ^{fg}	25.3 ^{fg}
202	283.1 ^{h}	455.0 ^{g}	94.4 ^{g}	151.6 ^{gh}	5176.6 ^{d}	5248.8 ^{f}	32.5 ^{f}	50.2 ^{d}	32.2 ^{d}	47.4 ^{c}	64.7 ^{f}	97.6 ^{de}	30.5 ^{df}	73.9 ^{df}
203	535.3 ^{bc}	661.0 ^{a}	178.5 ^{bc}	220.3 ^{a}	64.7.0 ^{h}	73.3 ^{kl}	2.4 ^{i}	3.0 ^{f}	2.1 ^{f}	2.5 ^{e}	4.6 ^{hi}	5.6 ^{gh}	4.0 ^{fg}	6.1 ^{g}
101/201	557.2 ^{b}	562.0 ^{bd}	185.7 ^{b}	187.5 ^{bc}	7726.6 ^{a}	7999.3 ^{a}	77.3 ^{d}	93.9 ^{c}	4.5 ^{ef}	5.5 ^{e}	81.8 ^{e}	99.4 ^{de}	76.0 ^{c}	93.2 ^{de}
101/202	547.6 ^{b}	549 ^{be}	182.5 ^{b}	179.8 ^{be}	5925.3 ^{c}	6338.2 ^{d}	123.6 ^{b}	160.5 ^{b}	12.1 ^{e}	21.8 ^{d}	135.8 ^{c}	182.4 ^{c}	123.5 ^{b}	164.1 ^{bc}
101/203	373.5 ^{fg}	568.0 ^{bc}	93.4 ^{g}	141.9 ^{h}	196.7 ^{h}	204.8 ^{jk}	93.7 ^{c}	110.3 ^{c}	2.3 ^{f}	3.5 ^{e}	96.1 ^{e}	113.9 ^{d}	44.8 ^{de}	80.8 ^{df}
102/201	433.6 ^{e}	655.0 ^{a}	144.5 ^{e}	211.6 ^{a}	6686.5 ^{b}	6943.2 ^{c}	24.2 ^{fg}	39.8 ^{de}	1.2 ^{f}	1.4 ^{e}	25.3 ^{g}	41.2 ^{fg}	18.3 ^{fg}	43.6 ^{eg}
102/202	360.8 ^{g}	482.0 ^{fg}	120.3 ^{f}	160.5 ^{fg}	5139.3 ^{d}	6273.0 ^{de}	116.9 ^{b}	146.7 ^{b}	1.7 ^{f}	3.1 ^{e}	118.6 ^{d}	149.9 ^{c}	71.4 ^{c}	120.4 ^{cd}
102/203	632.0 ^{a}	660.0 ^{a}	210.7 ^{a}	219.9 ^{a}	2396.9 ^{f}	1483.4 ^{i}	14.1 ^{gi}	16.6 ^{ef}	6.4 ^{ef}	7.3 ^{e}	20.5 ^{gh}	23.9 ^{gh}	21.6 ^{eg}	26.4 ^{fg}
103/201	422.9 ^{e}	517.0 ^{df}	141.0 ^{e}	172.5 ^{df}	5788.3 ^{c}	6136.4 ^{e}	17.8 ^{gh}	20.8 ^{ef}	3.5 ^{ef}	4.7 ^{e}	21.4 ^{gh}	25.5 ^{gh}	15.0 ^{fg}	22.0 ^{fg}
103/202	402.4 ^{ef}	523.0 ^{cf}	134.1 ^{e}	174.2 ^{cf}	4072.5 ^{e}	4472.2 ^{g}	1.7 ^{i}	2.4 ^{f}	0.6 ^{f}	0.9 ^{e}	2.4 ^{i}	3.3 ^{h}	1.6 ^{i}	2.8 ^{g}
103/203	511.5 c	576.0 ^{b}	170.5 ^{c}	192.0 ^{b}	231.7 ^{h}	298.6 ^{j}	2.6 ^{i}	3.7 ^{f}	2.3 ^{f}	2.5 ^{e}	4.9 ^{hi}	6.2 ^{gh}	4.2 ^{fg}	6.0 ^{g}
301	465.9 ^{d}	479.0 ^{fg}	159.1 ^{d}	161.9 ^{fg}	2199.6 ^{f}	2512.9 ^{h}	4.9 ^{hi}	5.2 ^{f}	3.5 ^{ef}	3.6 ^{e}	8.4 ^{gi}	8.8 ^{gh}	6.7 ^{fg}	7.1 ^{g}
CV (%)	4.4	4.7	4.4	4.6	4.0	3.0	9.0	14.0	10.7	14.1	10.7	12.1	17.3	24.6
F-test	**	**	**	**	**	**	**	**	**	**	**	**	**	**

** Significant at $p < 0.01$. Different superscript lower case letters indicate least significant differences within each column by Duncan's-test ($p < 0.05$).

However, the mean for sum CAPs content in 102/203 at KU was higher than that at KKU because genotype based on environment interaction was significant for this trait. Among parents, 203 was the highest for fresh yield (535.3 and 661.0 g/plant at KU and KKU, respectively), and 201 was the highest for sum CAPs (6616.3 and 7322.4 6 µg/g DW at KU and KKU, respectively), whereas 202 was the highest for sum CAPs (5176.6 and 5248.8 µg/g DW at KU and KKU, respectively). Moreover, 101 was highest for CAT (731.4 at KU and 924.9 µg/g DW at KKU), DI-CAT (508.4 at KU and 627.3 µg/g DW at KKU), sum CATs (1240.0 at KU and 1552.2 µg/g DW at KKU) and CAT yield (720.9 at KU and 1344.7 mg/plant at KKU). Among the crosses, 102/203 had the highest fresh yield (632.0 at KU and 660.0 g/plant at KKU), and 101/201 had the highest sum CAPs (7726.6 at KU and 7999.3 µg/g DW at KKU). In addition, the hybrid101/202 had high CAT (123.6 at KU and 160.5 µg/g DW at KKU), sum CATs (135.8 at KU and 182.4 µg/g DW at KKU) and CAT yield (123.5 at KU and 164.1 mg/plant at KKU), whereas the hybrid 102/202 had high CAT (116.9 at KU and 146.7 µg/g DW at KKU) and sum CATs (118.6 at KU and 149.9 µg/g DW at KKU).

3.2. General Combining Ability (GCA)

The GCA effects for all parents evaluated in the two environments are presented in Table 4. In this study, positive and high GCA values are preferable for most traits, except for sum CAPs, because the objective of this study was to increase CAT and reduce pungency. One or more parents had positive and significant GCA values for fresh yield, dry yield, Sum CAPs, CAT, DI-CAT, Sum CATs and CAT yield. The parents with good GCA effects at KU and KKU may not be the same because of significant GCA–E interaction. For fresh and dry yields, significant interactions were only presented for male GCA; the parent 203 had the highest GCA for fresh yield at KU (34.4 **) and KKU (35.4 **). The parent 203 also had the highest negative and significant GCA for sum CAPs at KU (−3298.7 **) and KKU (−3316.7 **). The parent 102 had the highest values of GCA for dry yield at KU and KKU, but it only had high and positive value of GCA for fresh yield at KKU, whereas the parent 101 only had high and positive value of GCA for fresh yield at KU. The parent 101 also had the highest values of GCA for CAT, DI-CAT, sum CATs and CAT yield at KU and KKU. The parent 202 had high values of GCA for CAT, DI-CAT, sum CATs and CAT yield at KU and KKU (except DI-CAT at KU), but it had negative and significant values of GCA for fresh yield (−34.4 ** at Ku and −47.9 at KKU) and dry yield (−8.0 ** at KU and −10.7 * at KKU) at both locations.

3.3. Specific Combining Ability (SCA)

A high and positive SCA effect is favorable for fresh and dry yields. The hybrids 101/202 and 102/203 showed consistently high and positive SCA effects for these traits across the two environments (Table 5). Other hybrids were 101/201, which showed good SCA effects for fresh weight and dry weight at KU, and 102/201, which showed good SCA effects at KKU.

A high and negative SCA effect is required for sum CAPs, and the most promising hybrids for these traits were 101/203 and 102/201, which showed negative, high and consistent SCA effects. The hybrid 102/202 also only had a good SCA effect at KU, whereas 103/202 only had a good SCA effect at KKU.

The hybrids with high and positive SCA effects are promising for CAT, DI-CAT, sum CATs and CAT yield. Based on these criteria, 103/201 and 103/203 were the best hybrids for these traits, and they also showed consistent performance in terms of SCA across the two environments. Unfortunately, this study did not find good SCA in terms of yield, sum CAPs, CAT, DI-CAT, sum CATs and CAT yield in the same hybrid.

Table 4. General combining ability (GCA) of six parental lines in terms of fruit yield and phytochemical traits determined at Kyoto University (KU), Japan, during the spring of 2015 and Khon Kaen University (KKU), Thailand, during the rainy season of 2016.

Parents	Fresh Yield (g/plant)		Dry Yield (g/plant)		Sum CAPs (µg/g DW)		CAT (µg/g DW)		DI-CAT (µg/g DW)		Sum CATs (µg/g DW)		CAT Yield (mg/plant)	
	KU	KKU	KU	KKU	KU	KKU	KU	KKU	KU	KKU	KU	KKU	KU	KKU
101	21.5 *	−5.9	0.2	−12.5 **	375.8	−911.0	45.8 **	55.5 **	2.5 **	4.6 **	48.3 **	60.2 **	39.6 **	50.6 **
102	4.2	33.0 *	4.9 **	15.1 **	500.5	920.9	−0.7	1.6	−0.7	−1.7	−1.5	−0.1	−4.7	1.3
103	−25.7 *	−27.1 *	−5.1 **	−2.6	−876.3	−9.9	−45.1 **	−57.1 **	−1.7 *	−2.9 *	−46.7 **	−60.1 **	−34.9 **	−51.9 **
201	−0.1	12.5	3.4	8.3 *	2493.4 **	3380.6 **	−12.7	−14.6	−0.8	−1.8	−13.5	−16.4	−5.4	−9.2
202	−34.4 **	−47.9 **	−8.0 **	−10.7 *	805.3	−63.9	28.3 *	37.1 *	1.0	3.0 *	29.3 *	40.1 *	23.7 *	33.6 *
203	34.4 **	35.4 **	4.6	2.4	−3298.7 **	−3316.7 **	−15.6	−22.5	−0.2	−1.2	−15.8	−23.7	−18.3 *	−24.4

** and * are significant at $p \leq 0.01$ and $p \leq 0.05$, respectively.

Table 5. Specific combining ability (SCA) of nine crosses in terms of fruit yield and phytochemical traits determined at Kyoto University (KU), Japan, during the spring of 2015 and Khon Kaen University (KKU), Thailand, during the rainy season of 2016.

The Crosses	Fresh Yield (g/plant)		Dry Yield (g/plant)		Sum CAPs (µg/g DW)		CAT (µg/g DW)		DI-CAT (µg/g DW)		Sum CATs (µg/g DW)		CAT Yield (mg/plant)	
	KU	KKU	KU	KKU	KU	KKU	KU	KKU	KU	KKU	KU	KKU	KU	KKU
101/201	64.5 **	−9.9 **	28.4 **	9.4 **	617.0 **	475.5 **	−8.2 **	−13.1 **	−1.0 **	−3.0 **	−9.3 **	−16.1 **	0.0	−10.3 **
101/202	89.2 **	37.4 **	36.6 **	20.8 **	503.8 **	146.2 **	−2.9	1.8	4.8 **	8.6 **	1.9	10.4 **	18.4 **	17.8 **
101/203	−153.7 **	−27.6 **	−65.0 **	−30.2 **	−1120.8 **	−621.7 **	11.1 **	11.3 **	−3.8	−5.6 **	7.3 **	5.7 *	−18.3 **	−7.5 **
102/201	−41.9 **	43.6 **	−17.4 **	5.9 **	−547.8 **	−299.7 **	−14.9 **	−13.3 **	−1.1 **	−0.8	−16.0 **	−14.1 **	−13.4 **	−10.7 **
102/202	−80.3 **	−69.3 **	−30.2 **	−26.1 **	−406.9 **	361.9 **	36.9 **	41.9 **	−2.4 **	−3.8 **	34.5 **	38.1 **	10.6 **	23.3 **
102/203	122.2 **	25.7 **	47.6 **	20.2 **	954.7 **	−62.2	−22.0 **	−28.6 **	3.5 *	4.6 **	−18.5 **	−24.0 **	2.8	−12.7 **
103/201	−22.6 **	−33.7 **	−11.0 **	−15.4 **	−69.2	−175.7 **	23.1 **	26.4 **	2.1 **	3.8 **	25.3 **	30.2 **	13.5 **	20.9 **
103/202	−8.9	31.8 **	−6.4 *	5.3 **	−96.9	−508.1 **	−34.0 **	−43.7 **	−2.5 **	−4.8 **	−36.5 **	−48.5 **	−29.0 **	−41.1 **
103/203	31.5 **	1.9	17.4 **	10.0 **	166.2 **	683.8 **	10.9 **	17.3 **	0.3	1.0*	11.1 **	18.3 **	15.6 **	20.1 **

** and * are significant at $p \leq 0.01$ and $p \leq 0.05$, respectively.

3.4. Correlation

Fresh and dry yields of pepper hybrids were inter-related with a correlation coefficient of 0.987 **, and the correlations between CAT, DI-CAT, sum CATs and CAT yield were also positive and significant, with the correlation coefficients ranging from 0.967 ** to 0.996 **. Sum CAPs was negatively and significantly correlated with CAT (−0.240 *), DI-CAT (−0.323 *), sum CATs (−0.276 *) and CAT yield (−0.248 *). Fresh and dry yields were not significantly correlated with sum CAPs, CAT, DI-CAT, sum CATs and CAT yield (Table 6).

Table 6. Correlations between fresh yield, dry yield, sum CAPs, CAT, DI-CAT and CAT yield of pepper hybrids evaluated in two environments in KU and KKU.

	Fresh Yield	Dry Yield	Sum CAPs	CAT	DI-CAT	Sum CATs
Dry yield	0.987 **					
Sum CAPs	0.180	0.217				
CAT	−0.071	−0.076	−0.240			
DI-CAT	−0.121	−0.108	−0.323	0.967 **		
Sum CATs	−0.091	−0.088	−0.276	0.994 **	0.989 **	
CAT yield	−0.053	−0.047	−0.248	0.994 **	0.981 **	0.996 **

** Significant at 0.01 probability level.

4. Discussion

The interaction between environment and variety for all traits studied indicated the different responses of different cultivars to different environments [20]. Chili plants exhibited sensitivity to many environmental conditions, hence leading to alterations in their metabolic composition [21]. For all traits, the cultivar was the largest contributor to variability observed in this study. This result suggests that the particular pepper cultivars with the highest fruit yield Sum CAPs and Sum CATs contents should be selected in the optimum environment to grow high-quality peppers [22–25]. The mean actual values for most traits in most cultivars under the KKU environment were higher than those under KU. This observation might be true because most of the cultivars used here were selected in the KKU area for many years. The air temperatures at KKU (33 °C and 23 to 24 °C day/night temperatures) were relatively uniform and similar to the great temperature for fruit set of peppers (Figure 1). Temperature strongly influenced flower and fruit development and yield in pepper [26]. In general, the fruit set of peppers were great at 28 to 32 °C and 18 to 26 °C day/night temperatures [27–29]. Moreover, the Sum CAPs and Sum CATs had high contents in the KKU environment (200 m MASL), as this environment is at a higher elevation than the KU environment (50 m MASL). These results might be attributed to the elevation strongly influencing Sum CAPs contents in medium- and high-pungency varieties, as some varieties showed high levels of Sum CAPs at high elevation [30]. In contrast, the Sum CAPs content of 102/203 was different between the KU and KKU environments, with the highest Sum CAPs content produced in the KU environment. Therefore, this cross is considered to be good for a specific environment, i.e., the KU environment. This result might be attributed to its low pungency (<50,000 SHU.), which is unstable in a different environment [31]. This cultivar is considered to be good for specific adaptation in a high Sum CAPs-accumulating environment. Variations in the yields of Sum CAPs and Sum CATs in this population can be exploited by selecting parental lines or the crosses with high Sum CAPs and Sum CATs yields to grow in an optimal environment.

Combining ability studies show the occurrence of both additive and non-additive gene effects in this study. The high proportion percentages of GCA for Sum CAPs, CAT, Sum CATs and CAT yield were greater than that of SCA. This finding indicated that the importance of the additive gene effect was the main effect on these traits. The high values for fruit yield were found in the line 203, for Sum CATs were found in the line 101 and for Sum CAPs were found in the line 201, with positive GCA effects; these three cultivars might be considered to be good parents, which can pass on these target traits into their offspring [32,33]. Statistically high GCA effects with negative values were estimated for some cultivars, meaning that the cultivars would pass on to their progeny the tendency of low productivity of Sum CAPs and Sum CATs traits [34].

The high proportion percentage of SCA revealed that the non-additive gene effect was the main effect on fresh yield, dry yield and DI-CAT [20]. The relative contribution of individual parents to improving the specific trait in the population can be estimated by comparison to the GCA effects [32]. The cross 102/203 showing high positive SCA values for fresh and dry yield involved parents with high/high GCA. The best cross for Sum CAPs (101/201) was derived from parents with high/low GCA. The best cross for CAT (102/202), DI-CAT (101/202), Sum CATs (102/202) and CAT yield (102/202) traits were derived from parents with low/high GCA, high/high GCA and high/low GCA, respectively. This result revealed that the best cross with high positive SCA values was not always produced by the parents with high positive GCA values [35].

In general, pepper cultivars with high CAT yields are ideal for CAT production. It is interesting to note that the cross 101/202 presented the highest actual value of CAT yield with high positive SCA effect, which was found in the lines 101 and 202 and useful for the prediction of the introgression of high CAT yields into offspring based on the high GCA values of both parents. Remarkably, 'KKU-P11003' (202),which was one of the improved varieties at KKU, showed the highest positive GCA value for all capsaicinoids. This result revealed that the crosses with high capsaicinoids were always produced by 'KKU-P11003′. Moreover, this cultivar presented the values of narrow-sense heritability (h_n^2) differently between capsaicin (0.32), dihydrocapsaicin (0.92) and capsaicinoids (0.62) [33]. Considering the relationship between the inheritance of CAPs and CATs in 202, this cultivar was considered to be a good parent, which can pass on CATs content, as well as CAPs, to the offspring. This finding is due to CATs sharing closely related structures with CAPs, but the putative-aminotransferase (*p-AMT*) gene mutation determined CATs biosynthesis [11,36,37]. Thus, due to the medium-to-high actual value for CAT yield in lines 101 and 202 and the high positive GCA effect of both parents, the best cross between the lines 101 and 202 was suggested to produce F_1 hybrid.

As a result, all of the crosses presented low mean actual values (<500 μg/g DW) for CAT, DI-CAT and Sum CATs that were much lower than those of their parents [1,12,35]. Moreover, this result may imply that recessive gene controlling occurred in CAT, DI-CAT and Sum CATs traits [11,12,14]. However, the quantitative control of CATs in pepper was not elucidated. Thus, it is possible that CATs traits could be synthesized by different genes via phenylpropanoid pathways, such as *pAMT*, *C4H*, *4CL* and *CSE*, on different positions in the genome [11,36,38,39]. Based on the low actual mean of Sum CATs in these two crosses, the backcross method would be a good breeding method to increase yield and Sum CATs levels in the population studied [40,41]. Moreover, the two crosses, i.e., 101/201 and 101/202, might be good for developing the high-pungency cultivars due to their high SCA and high actual mean value of Sum CAPs. Considering the high actual mean of Sum CAPs in these two crosses over their parents, it might be possible to produce crosses with high pungency and yields [42,43]. The genetic makeup of Sum CAPs content has been reported to be affected by the genotype and environment, with additive gene effects [31,44–46]. Thus, a stability analysis is required for testing in multiple environments. However, six parental lines originate from a small number of cultivars and close genetic variation (narrow genetic base) for Sum CATs traits due to the lack of information on the variation in Sum CATs content (*C. annuum* L.) germplasm, and some of the genetic resources were not available. Consequently, these population materials could not produce the high Sum CATs contents with no Sum CAPs cultivars compared to commercial cultivars, like 'CH-19 sweet' [47]. The results found in this study would be of benefit to plant breeders, although this study was primarily conducted to estimate the combining ability and gene actions of Sum CATs and its component. Furthermore, broad genetic base population for high Sum CATs traits or interspecific hybridization program should be used to improve new pepper cultivars with high contents of Sum CATs and its components.

5. Conclusions

Environment greatly affected fresh fruit and dry fruit yields of pepper, and it also affected their phytochemical contents to a lesser extent. The most important environmental factor affecting the performance of pepper would be temperature because KU is located in a temperate region, while KKU is located in a tropical region, although the crop was planted in the same months of different years. The ideal parent with good GCA effects for all desired characters could not be identified in this study. However, the parents with good GCA effects for individual characters could be identified. Parent 102 had high and positive GCA effects for fresh fruit and dry fruit yields. Parent 203 was the best parent for the lowest and negative GCA effects for sum CAPs at KU and KKU. Parent 101 had high and stable GCA effects for CAT, sum CATs, DI-CAT and CAT yield across both environments. Parent 101/203 was the best hybrid in terms of the lowest SCA effect for sum CAPs across the two environments, whereas 103/201 and 103/203 had consistently high and positive SCA effects for CAT, DI-CAT, sum CATs and CAT yield across the two environments.

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References

1. Tanaka, Y.; Yoneda, H.; Hosokawa, M.; Miwa, T.; Yazawa, S. Application of marker-assisted selection in breeding of a new fresh pepper cultivar (*Capsicum annuum*) containing capsinoids, low-pungent capsaicinoid analogs. *Sci. Hortic.* **2014**, *165*, 242–245. [CrossRef]
2. Fayos, O.; Savirón, M.; Orduna, J.; Barbero, G.; Mallor, C.; Garcés-Claver, A. Quantitation of capsiate and dihydrocapsiate and tentative identification of minor capsinoids in pepper fruits (*Capsicum* spp.) by HPLC-ESI-MS/MS(QTOF). *Food Chem.* **2019**, *270*, 264–272. [CrossRef] [PubMed]
3. Sharma, S.K.; Vij, A.S.; Sharma, M. Mechanisms and clinical uses of capsaicin. *Eur. J. Pharmacol.* **2013**, *720*, 55–62. [CrossRef] [PubMed]
4. Singh, N.; Majumder, S.; Singh, S.; Vikram, P. Genetic diversity in Indian rice germplasm set using phenotypic andgenotypic variables simultaneously. *Indian J. Genet. Plant Breed.* **2016**, *76*, 246–254. [CrossRef]
5. Basbag, S.; Ekinci, R.; Gencer, O. Combining ability and heterosis for earliness characters in line × tester population of *Gossypium hirsutum* L. *Hereditas* **2007**, *144*, 185–190. [CrossRef]
6. Acquaah, G. *Principles of Plant Genetics and Breeding*; Malden Blackwell Publishing: Oxford, UK, 2007.
7. Barnard, A.D.; Labuschagne, M.T.; van Niekerk, H.A. Heritability estimates of bread wheat quality traits in the Western Cape province of South Africa. *Euphytica* **2002**, *127*, 115–122. [CrossRef]
8. Pandey, S.; Singh, J.; Updhaya, A.K.; Ram, D.; Rai, M. Ascorbate and carotenoid content in Indian collection of pumpkin (*Cucurbita moschata* Duch. ex Poir.). *Cucurbit Genet. Coop. Rep.* **2003**, *26*, 51–53.
9. Zewdie, Y.; Bosland, P.W. Capsaicinoid inheritance in an interspecific hybridization of *Capsicum annuum* × *C. chinense*. *J. Am. Soc. Hortic. Sci.* **2000**, *125*, 448–453. [CrossRef]
10. Ohta, Y. Physiological and genetical studies on the pungency of Capsicum. IV. Secretory organs, receptacles and distribution of capsaicin in the Capsicum fruit. *Jpn. J. Breed.* **1962**, *12*, 179–183. [CrossRef]
11. Lang, Y.; Kisaka, H.; Sugiyama, R.; Nomura, K.; Morita, A.; Watanabe, T.; Tanaka, Y.; Yazawa, S.; Miwa, T. Functional loss of pAMT results in biosynthesis of capsinoids, capsaicinoid analogs, in *Capsicum annuum* cv. CH-19 Sweet. *Plant J.* **2009**, *59*, 953–961. [CrossRef]

12. Tanaka, Y.; Hosokawa, M.; Miwa, T.; Watanabe, T.; Yazawa, S. Newly mutated putative-aminotransferase in nonpungent pepper (*Capsicum annuum*) results in biosynthesis of capsinoids, capsaicinoid analogues. *J. Agric. Food Chem.* **2010**, *58*, 1761–1767. [CrossRef] [PubMed]
13. Sung, J.H. Genetic Analysis of Capsinoid in *Capsicum annuum*. Master's Thesis, Seoul National University, Seoul, Republic of Korea, 2007.
14. Han, K.; Jeong, H.-J.; Sung, J.; Keum, Y.S.; Cho, M.-C.; Kim, J.-H.; Kwon, J.-K.; Kim, B.-D.; Kang, B.-C. Biosynthesis of Capsinoids is controlled by the Pun1 locus in pepper. *Mol. Breed.* **2013**, *31*, 537–548. [CrossRef]
15. Tarinta, T.; Chanthai, S.; Lertrat, K.; Nawata, E.; Techawongstien, S. Novel source of the secondary metabolite capsiate in Capsicum germplasm accession. *SABRAO J. Breed. Genet.* **2020**, *52*, 144–157.
16. Tanaka, Y.; Hosokawa, M.; Otsu, K.; Watanabe, T.; Yazawa, S. Assessment of Capsiconinoid composition, nonpungent capsaicinoid analogues, in Capsicum cultivars. *J. Agric. Food Chem.* **2009**, *57*, 5407–5412. [CrossRef]
17. Gomez, K.A.; Gomez, A.A. *Statistical Procedures for Agricultural Research*, 2nd ed.; Wiley: New York, NY, USA, 1984.
18. Hallauer, A.R.; Carena, M.J.; Filho, J.B. *Quantitative Genetics in Maize Breeding*; Springer: New York, NY, USA, 2010; Volume 3.
19. Singh, R.K.; Chaudhary, B.D. *Biometrical Methods in Quantitative Genetic Analysis*; Kalyani Publishers: Delhi, India, 1958.
20. Griffing, B. Concept of general and specific combining ability in relation to diallel crossing systems. *Aust. J. Biol. Sci.* **1956**, *9*, 463–493. [CrossRef]
21. Zamljen, T.; Jakopič, J.; Hudina, M.; Veberič, R.; Slatnar, A. Infuence of intra and inter species variation in chilies (*Capsicum* spp.) on metabolite composition of three fruit segments. *Sci. Rep.* **2021**, *11*, 4932. [CrossRef]
22. Bernal, M.A.; Calderón, A.A.; Ferrer, M.A.; De Cáceres, F.M.; Barceló, A.R. Oxidation of Capsaicin and Capsaicin Phenolic Precursors by the Basic Peroxidase Isoenzyme B6 from Hot Pepper. *J. Agric. Food Chem.* **1995**, *43*, 352–355. [CrossRef]
23. Estrada, B.; Bernal, M.; Díaz, J.; Pomar, F.; Merino, F. Fruit development in *Capsicum annuum*: Changes in capsaicin, lignin, free phenolics, and peroxidase patterns. *J. Agric. Food Chem.* **2000**, *48*, 6234–6239. [CrossRef]
24. Russo, V.M.; Biles, C.L. Fertilizer rate and β-galactosidase and peroxidase activity in pepper fruit at different stages and years of harvest. *Plant Foods Hum. Nutr.* **2004**, *58*, 231–239. [CrossRef]
25. Jeeatid, N.; Suriharn, B.; Techawongstien, S.; Chanthai, S.; Bosland, P.W.; Techawongstien, S. Evaluation of the effect of genotype-by-environment interaction on capsaicinoid production in hot pepper hybrids (*Capsicum chinense* Jacq.) under controlled environment. *Sci. Hortic.* **2018**, *235*, 334–339. [CrossRef]
26. Polowick, P.L.; Sawhney, V.K. Temperature effects on male fertility and flower and fruit development in *Capsicum annuum* L. *Sci. Hortic.* **1985**, *25*, 117–127. [CrossRef]
27. Aloni, B.; Peet, M.; Pharr, M.; Karni, L. The effect of high temperature and high atmospheric CO_2 on carbohydrate changes in bell pepper (*Capsicum annuum*) pollen in relation to its germination. *Physiol. Plant.* **2001**, *112*, 505–512. [CrossRef] [PubMed]
28. Erickson, A.N.; Markhart, A.H. Flower developmental stage and organ sensitivity of bell pepper (*Capsicum annuum* L.) to elevated temperature. *Plant Cell Environ.* **2002**, *25*, 123–130. [CrossRef]
29. Wubs, A.M.; Heuvelink, E.; Marcelis, L.F.M. Abortion of reproductive organs in sweet pepper (*Capsicum annuum* L.): A review. *J. Hortic. Sci. Biotechnol.* **2009**, *84*, 467–475. [CrossRef]
30. Gurung, T.; Techawongstien, S.; Suriharn, B.; Techawongstien, S. Impact of environments on the accumulation of capsaicinoids in Capsicum spp. *HortScience* **2011**, *46*, 1576–1581. [CrossRef]
31. Gurung, T.; Techawongstien, S.; Suriharn, B.; Techawongstien, S. Stability analysis of yield and capsaicinoids content in chilli (*Capsicum* spp.) grown across six environments. *Euphytica* **2012**, *187*, 11–18. [CrossRef]
32. Lippert, L.F. Heterosis and combining ability in Chili peppers by diallel analysis. *Crop. Sci.* **1975**, *15*, 323–325. [CrossRef]
33. Tempeetikul, V.; Techawongstien, S.; Lertrat, K.; Techawongstien, S. Inheritance of pungency in Thai hot pepper (*Capsaicum annuum* L.). *SABRAO J. Breed. Genet.* **2013**, *45*, 130–143.
34. Nyadanu, D.; Lowor, S.T.; Akrofi, A.Y.; Adomako, B.; Dzahini-Obiatey, H.; Akromah, R.; Awuah, R.T.; Kwoseh, C.; Adu-Amoah, R.; Kwarteng, A.O. Mode of inheritance and combining ability studies on epicuticular wax production in resistance to black pod disease in cacao (*Theobroma cacao* L.). *Sci. Hortic.* **2019**, *243*, 34–40. [CrossRef]
35. Shukla, S.K.; Pandey, M.P. Combining ability and heterosis over environments for yield and yield components in two line hybrids involving thermosensitive genic male sterile lines in rice (*Oryza sativa* L.). *Plant Breed.* **2008**, *127*, 28–32. [CrossRef]
36. Stewart, C.; Mazourek, M.; Stellari, G.M.; O'Connell, M.; Jahn, M. Genetic control of pungency in *C. chinense* via the Pun1 locus. *J. Exp. Bot.* **2007**, *58*, 979–991. [CrossRef] [PubMed]
37. Sasahara, I.; Furuhata, Y.; Iwasaki, Y.; Inoue, N.; Sato, H.; Watanabe, T.; Takahashi, M. Assessment of the biological similarity of three capsaicin analogs (Capsinoids) found in non-pungent chili pepper (CH-19 Sweet) fruits. *Biosci. Biotechnol. Biochem.* **2010**, *74*, 274–278. [CrossRef] [PubMed]
38. Jang, S.; Han, K.; Jo, Y.D.; Jeong, H.J.; Siddique, M.I.; Kang, B.C. Substitution of a Dysfunctional pAMT Allele Results in Low-Pungency but High Levels of Capsinoid in *Capsicum chinense* 'Habanero'. *Plant Breed. Biotechnol.* **2015**, *3*, 119–128. [CrossRef]
39. Stewart, C.; Kang, B.C.; Liu, K.; Mazourek, M.; Moore, S.L.; Yoo, E.Y.; Kim, B.D.; Paran, I.; Jahn, M.M. The Pun1 gene for pungency in pepper encodes a putative acyltransferase. *Plant J.* **2005**, *42*, 675–688. [CrossRef]
40. Veni, B.K.; Rani, N.S. Combining ability studies for important physico-chemical quality characteristics in aromatic rice. *J. Res. Angrau.* **2007**, *35*, 13–20.

41. Zewdie, Y.; Bosland, P.W.; Steiner, R. Combining ability and heterosis for capsaicinoids in Capsicum pubescens. *HortScience* **2001**, *36*, 1315–1317. [CrossRef]
42. Deshpande, R.B. Studies in Indian chillis: Inheritance of pungency in *Capsicum annuum* L. *Indian J. Agric. Sci.* **1935**, *5*, 513–516.
43. Garcés-Claver, A.; Gil-Ortega, R.; Ivarez-Fernández, A.; Arnedo-Andrés, M.S. Inheritance of capsaicin and dihydrocapsaicin, determined by HPLC-ESI/MS, in an intraspecific cross of *Capsicum annuum* L. *J. Agric. Food Chem.* **2007**, *55*, 6951–6957. [CrossRef]
44. Estrada, B.; Bernal, M.A.; Díaz, J.; Pomar, F.; Merino, F. Capsaicinoids in vegetative organs of *Capsicum annuum* L. in relation to fruiting. *J. Agric. Food Chem.* **2002**, *50*, 1188–1191. [CrossRef]
45. Phimchan, P.; Techawongstien, S.; Chanthai, S.; Bosland, P.W. Impact of drought stress on the accumulation of capsaicinoids in Capsicum cultivars with different initial capsaicinoid levels. *HortScience* **2012**, *47*, 1204–1209. [CrossRef]
46. Suwor, P.; Thummabenjapone, P.; Sanitchon, J.; Kumar, S.; Techawongstien, S. Phenotypic and genotypic responses of chili (*Capsicum annuum* L.) progressive lines with different resistant genes against anthracnose pathogen (*Colletotrichum* spp.). *Eur. J. Plant Pathol.* **2015**, *143*, 725–736. [CrossRef]
47. Yazawa, S.; Suetom, N.; Okamoto, K.; Namiki, T. Content of capsaicinoids and capsaicinoids-like substances in fruit of pepper (*Capsicum annuum* L.) hybrids made with 'CH-19 sweet' as a parent. *J. Jpn. Soc. Hortic. Sci.* **1989**, *58*, 601–607. [CrossRef]