

Combining Ability and Heritability for Capsaicinoid Content in Field-Grown Tunisian Hot Pepper Varieties (*Capsicum annuum* L.)

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Abstract – In Tunisia, pepper (*Capsicum* spp.) is consumed for its fruits, which are used either fresh or dried. The genetic resources of pepper are important as a natural source of capsaicinoids, which confer hotness to its fruits. Fifteen F1 hybrids and six parental genotypes were used to assess combining ability and heritability for capsaicin, dihydrocapsaicin, nordihydrocapsaicin and total capsaicinoid in green pepper fruits (*Capsicum annuum* L.). The mean squares for general (GCA) and specific (SCA) combining abilities were highly significant for these characters, suggesting the importance of both additive and non-additive gene effects. However, additive gene action was more important, as GCA estimates were much higher than SCA effects. Among the parental genotypes, ‘Rouge Long’ and ‘Piment Sesseb’ and ‘Chaabani’ were good general combiners for capsaicin, dihydrocapsaicin, nordihydrocapsaicin and total capsaicinoid, respectively, and could be used to improve these traits in pepper breeding programmes for the accumulation of favorable genes. They also showed the highest per se performance (0.67mg.g⁻¹, 1.09 µg.g⁻¹, 0.32 mg.g⁻¹ and 2.08 mg.g⁻¹ respectively) for these quality traits. The narrow sense heritability estimate for total capsaicinoid, was relatively high (45%) indicating that the environment had a less pronounced effect on this trait.

Keywords – Pepper Fruits, Capsaicinoids, General and Specific Combining Ability, Heritability.

Abbreviations – SCA, Specific Combining Ability; GCA, General Combining Ability; H²_b, Broad Sense Heritability; H²_n, Narrow Sense Heritability; H, High Parent Heterosis.

I. INTRODUCTION

Pepper (*Capsicum* spp.) is one of the most cultivated vegetable and spice crops in the world and alkaloid compounds that are synthesized and accumulated in pepper fruit are widely used in the food, medical, and pharmaceutical industries (Bosland and Votava, 2000, Caterina et al. 2000). In Tunisia, peppers are mainly consumed for the flavor and pungency of their fruits, which are used either fresh or dried.

Hot pepper fruits produce unique alkaloid compounds known capsaicinoids (Kobata et al., 1998) responsible of the pungent sensation in fruits of the genus *Capsicum* (Cinesro-Pineda et al., 2007). Pungency is caused mainly by five alkaloids: capsaicin, dihydrocapsaicin,

homocapsaicin, homodihydrocapsaicin and nordihydrocapsaicin. Of these, capsaicin (8-methyl-N-vanillyl-trans-6-nonenamide) and dihydrocapsaicin (8-methyl-N-vanillylnonamide) are responsible for 90% of total pungency (Govindarajan and Satyanaryana, 1991; Vázquez-Flota et al., 2007). All of these alkaloids are biosynthesized from L-phenylalanine and valine in the placenta by the condensation of vanillylamine with a short-chain branched fatty acid (Fujiwake et al., 1982a). The different forms of natural capsaicinoids depend on the number of lateral carbons chain or on the degree of unsaturation that is present (Ravishankar et al., 2003; Cruz-Pérez et al., 2007; Reyes-Escogido et al., 2011), mainly in the cell vacuole (Blum et al., 2003). The length of the alkyl chain influences pungency. Hence, lateral chain length is important for the bioactivity of capsaicinoids, which was higher at nine carbons atoms (Barbero et al., 2010).

The amount of capsaicinoids that accumulate in the placenta depends on the environment, genotype and their interaction (Zewdie and Bosland, 2000). The accumulation of capsaicinoids varies among genotypes (Ruiz-Lau et al., 2011) and fluctuates during the stages of pepper fruit development (Contreras-Padella and Yahia, 1998; Ben Mansour-Gueddes et al., 2012). In addition, Harvell and Bosland (1997) noted that the phenotypic expression of pungency is the result of a genotype-by-environment interaction. In general, capsaicin content is lower in cooler season than in warmer ones (Cotter, 1980). Drought and high temperature during the night causes capsaicinoids synthesis (Butnariu and Samfira, 2013). Therefore, high temperature and nutrient solution formulation had important effects on capsaicin contents (Rahman and Inden, 2012).

Early studies using organoleptic tests found that the presence/absence of pungency was controlled by a single dominant gene originally named Pun1. This gene has been mapped on chromosome two of the genus *Capsicum* and is essential for the control of capsaicinoid production; the allele for pungency Pun1 is dominant over the non-pungent one (Blum et al., 2002; Lefebvre et al., 2002; Stewart et al., 2005).

There is a large natural variability in capsaicinoid content in pepper genotypes and it constitutes a critical

component in breeding and production of this species (Garcés-Claver, 2007). Many authors have studied the genetic mechanism underlying the inheritance of pungency but this mechanism is still poorly understood. Ishikawa et al. (1995) reported 10 times greater capsaicinoid content in hybrid progeny than the most pungent parent. On the other hand, Understanding the mode of inheritance for individual capsaicinoids would enable breeders to manipulate and obtain balanced capsaicinoid profiles more effectively (Zewdie and Bosland, 2000). When studying the inheritance of both capsaicin and dihydrocapsaicin in an intraspecific cross of *C. annuum*, Garcés-Claver et al. (2007) reported that the type of gene action varied between these two compounds. Estimates of such gene action within a breeding population are important for determining which breeding procedure will efficiently improve the performance of the traits of interest (Geleta and Labuschagne, 2006).

The main objective of this study was to investigate the combining ability and heritability of capsaicin, dihydrocapsaicin, nordihydrocapsaicin and total capsaicinoid content assessed by high-performance liquid chromatography (HPLC), and to identify parental genotypes with good general combining ability (GCA) for these traits in Tunisian varieties of pungent peppers cultivated in the field. To avoid variability due to fruit maturation stage (Ben-Mansour et al. 2012), we assessed capsaicinoid compounds in mature green fruits. Better understanding the mode of inheritance of individual capsaicinoid compounds as well as the selection of better parents and hybrids are key factors to improve hot pepper breeding programs.

II. MATERIALS AND METHODS

Plant material and growth conditions

A half-diallel was created using six parent pepper varieties: Rouge Long, Piment Sesseb, Msarreh, Baklouti Essahel, Chaabani and Baklouti Kairouan. These pungent Tunisian varieties are representative landraces of Teboulba (35°38' N, 10°57' E), Sesseb (36°04' N, 10°12' E) SidiBouزيد Est (35°03' N, 9°35' E), Bekalta (35°36' N, 10°59' E), Chbikha (35°37' N, 9°55' E), Sbikha (35°55' N, 10°01' E), respectively (Table 1). These varieties were first propagated in a greenhouse by self-pollination to ensure genotypic stability for three generations. Parent plants were evaluated to determine the individual and total capsaicinoid content of mature green fruits and to test the uniformity of the capsaicinoid profile for each line before hybridization (Table 1).

Thirteen seedlings of six parent varieties were transplanted in the field in summer of 2006 in three replications. At the time of transplanting, the seedlings had six true leaves. Five plants were used as female parents to produce seeds from the direct cross hybrids. All of the crosses were performed plant to plant without mixing pollen. To this end, the floral buds of the female plants were covered with paper bags to prevent undesirable crosses. In each case, the plants were labeled with their identification number and the date of the cross.

Table 1: Description of the six parental lines of hot pepper used in this study. Six parental lines were selected based on their diverse genetic backgrounds and pungency levels for diallel crosses.

Code	Population Designation	Province of Origin	Pungency Level
P1	BakloutiEssahel	Sahel(Bekalta)	moderately pungent
P2	Rouge Long	Sahel(Teboulba)	very pungent
P3	Chaabani	Kairouan(Chebika)	moderately pungent
P4	Msarreh	SidiBouزيد Est	very pungent
P5	Baklouti Kairouan	Kairouan (Sbikha)	low pungent
P6	Piment Sesseb	Kairouan(Sesseb)	very pungent

Fifteen half-diallelF₁ crosses were successfully obtained. The six parents and the 15 F₁hybrids were evaluated in a randomized complete block design with three replications. Ten plants per genotype were planted in an open field in summer 2007 in well-prepared soil with 3plants m⁻². Fertilization and irrigation were provided as needed (Chaux et Foury, 1994). Since, the temperature affects the amount of capsaicinoids, Day and night temperatures and relative humidity were recorded daily during culture using a thermohydrograph (Fig. 1)

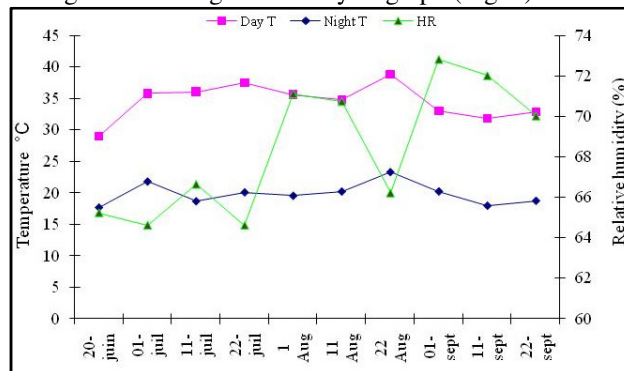


Fig.1. Evolution of day and night temperatures and relative humidity (HR) during the experiment. Climatic factors, namely night and day temperatures (T) and relative humidity (HR) were recorded during summer 2007 in an open field.

Extraction and quantification of capsaicinoids

Fifteen green fruits were harvested at the mature green stage from each genotype. Fruits were oven-dried at 60°C for 2 to 3 days until reaching constant weight, and ground to 1 mm for capsaicinoid analysis. Capsaicinoids were extracted and quantified according to the modified method of Collins et al. (1995). Two grams of dried ground pepper samples were extracted in 20 mL of acetonitrile and incubated in a water bath during 4 h at 80°C with regular shaking (every 30 min). After cooling and centrifugation, the supernatant of each extract was filtered through a 0.45 µm nylon filter prior to High Performance Liquid Chromatography (HPLC) analysis. Capsaicin, dihydrocapsaicin and nordihydrocapsaicin were quantified using

a HPLC analytical system (Waters, Milford, MA, USA). Elution was performed at 35°C with a flow rate of 1 mL min⁻¹ with the following gradient of eluant A (10% methanol) and eluant B (100% methanol): from 0 to 10 min, 43% A and 57% B; from 10 to 20 min, 32% A and 68% B, and from 20 to 30 min, 43% A and 57% B, for a total of 30 min. The capsaicinoids were separated on a Nova-Pak C18 4µm column (Waters, USA) and detected on a Scanning Fluorescence detector (Model 474 from Waters, USA). Excitation wavelength was set at 280 nm and emission wavelength at 338 nm. Retention times and quantities of capsaicin and dihydrocapsaicin were estimated by reference to standards (Sigma-Aldrich Co., St. Louis, MO). Retention times for nordihydrocapsaicin, capsaicin and dihydrocapsaicin were of 16.32, 16.94 and 20.08 min respectively. The concentration of nordihydrocapsaicin was expressed as mg. g⁻¹ dry weight (DW) equivalent capsaicin.

Statistical analysis

Half-diallel analysis of variance was performed using Griffing's method 2 model II for fixed genotypes (Griffing, 1956). The analysis was executed using the diallel-SAS program. The GCA/SCA ratio was determined for all traits. Narrow and broad sense heritability of these traits was estimated by applying variance analysis as follow:

$$\sigma_g^2 = 1/p + 2((CM_g - CM_s)) \quad (1)$$

and $\sigma_s^2 = CM_s - CM_e$ with CM_g , CM_s and CM_e respectively, mean square of parents, hybrids and error, p = number of parents.

The relative contributions of genetic components were determined to obtain estimates of GCA variance (σ_g^2) and SCA variance (σ_s^2) for each capsaicinoid.

Estimates of variance components due to general (σ_g^2) and specific (σ_s^2) combining abilities are used as indicators of additive (δ_A^2) and dominance (δ_D^2) variances.

The variance explained by the general combining ability effects of parents is a quarter of additive genetic variance: $\delta_A^2 = 4\sigma_g^2$.

The variance explained by the female and male interactions (specific combining ability) is one quarter of the dominance genetic variance: $\delta_D^2 = 4\sigma_s^2$.

Heritability expresses the proportion of the total variance that is attributable to the average effects of genes. Broad and narrow sense heritability was estimated based on the variance components. Therefore broad (H_b) and narrow (H_n) sense heritability were estimated for all capsaicinoids using the following equations developed by

Isik (2009); the percent heritability values were obtained using the following equation:

$$H_b^2 = [\delta_G^2 / \delta_P^2] * 100 \text{ or } \delta_P^2 = 2\sigma_g^2 + \sigma_s^2 + \sigma_e^2; \delta_G^2 = 4*(\sigma_g^2 + \sigma_s^2). \quad (2)$$

$$H_n^2 = [\delta_A^2 / \delta_P^2] * 100 \quad (3)$$

Where, σ_g^2 = Variance due to GCA; σ_s^2 = variance due to SCA; σ_e^2 = environmental variance; δ_G^2 is the total genotypic variance and δ_P^2 is the phenotypic variance.

The variance of general combining ability (δ_G^2) is multiplied by 2 because females and males contribute 1/4 of additive genetic variance to the total variance (Isik, 2009).

Duncan's new multiple range test (mean separation) was performed using the SAS program. The parents vs. crosses were tested for significance by using the CONTRAST statement in SAS (1999). The parents vs crosses comparison is a test for heterosis (average heterosis). For practical reasons, high parent heterosis was also calculated using the following formula and discussed: High parent heterosis = $\{[(F_1 - HP)/HP]*100\}$; where HP= high parent mean respective to the F₁ progeny being measured (Zewdie and Bosland, 2001).

III. RESULTS AND DISCUSSION

We report in this study the results on combining ability, heritability and heterosis of individual capsaicinoid compounds (capsaicin, dihydrocapsaicin, nordihydrocapsaicin) and total capsaicinoids in order to better understand the mode of inheritance of each compound separately. This issue is of up most importance because, in addition to the major interest in total capsaicinoid content related to the hotness of the fruits, new uses for pepper are emerging. For instance, fermented pepper has recently been shown to inhibit fat accumulation and improve lipid metabolism in mice (Yeon et al. 2013). However, the use of capsaicinoids as food additives and medicines is prevented by their high pungency and irritant effects. It is well known that 90% of pungency comes from capsaicin and dihydrocapsaicin (Govindarajan et al., 1987). While glycosides of both capsaicin and dihydrocapsaicin have potential anti-obesity effects (Katsuragi et al. 2010). The comprehension of the mode of inheritance of each compound will enable breeders to effectively manipulate capsaicinoid profiles.

Estimates of variance components and heritability

Highly significant genotypic differences were observed for capsaicinoids (Table 2).

Table 2: Mean squares from the half-diallel analysis for general combining ability (GCA) and specific combining ability (SCA) for capsaicin (CAP), dihydrocapsaicin (DH), nordihydrocapsaicin (NDH) and total capsaicinoid (CAPT) in six pepper genotypes and their hybrids.

Source	df [#]	CAP	DH	NDH	CAPT
Replication	2	1510.87 ^{ns}	1322.45 ^{ns}	115.07 ^{ns}	6485.15 ^{ns}
Parents	20	353168.37 ^{***}	416100.50 ^{***}	24546.79 ^{***}	1778134.75 ^{***}
GCA	5	485679.16 ^{***}	808085.30 ^{***}	59248.78 ^{***}	3124229.20 ^{***}
SCA	15	308998.11 ^{***}	285438.87 ^{***}	12979.46 ^{***}	1329436.60 ^{***}
Error	40	2609.67 ^{***}	3618.41 ^{***}	436.81 ^{***}	11684.48 ^{***}

GCA/SCA	1.6	2.8	4.6	2.4
H ² _n	24	55	77	45
H ² _b	84	95	93	95
C.V	7.5	9.2	16.2	7.4

#df = degree of freedom

*** Significance of the F statistic at the 0.001 probability level.

¶ns, indicates non-significance at P = 0.05.; CV = Coefficient of variation.

The contributions of GCA and SCA to genotypic variation were significant at the 1% level for capsaicin, dihydrocapsaicin, nordihydrocapsaicin and total capsaicinoids, indicating a wide range of variability among the parents. Parents vs crosses were also significantly different for all capsaicinoid contents. The significant mean squares observed for all the traits indicate a considerable variability in the parental lines as well as among the crosses. The magnitude variation of GCA and SCA indicates the importance of the additive and non-additive gene action in the inheritance of these traits, respectively (Table 2). Similar results were reported for total capsaicinoid content in *C. pubescens* crosses (Zewdie and Bosland, 2001).

The diversity in parental inbred lines produced a large amount of variability in progeny crosses. GCA explains genotypic variance due to additive genetic effects, and SCA explains the genotypic variance of dominance or epistasis. The proportion of additive effects compared to non-additive effects can be estimated by the GCA/SCA ratio (Sharma et al., 1991). The GCA/SCA ratio was high for capsaicin (1.6), dihydrocapsaicin (2.8), nordihydrocapsaicin (4.6) and total capsaicinoids (2.4), indicating the predominance of additive gene action in the inheritance of capsaicinoid compounds (Table 2). These high GCA/ASC ratios demonstrate that genetic variability in the F₁ generation is mainly due to the additive gene effect existing in the different parents (Biabani et al., 2012).

High broad sense heritability indicates genetic variance with a low influence of the environment, whereas low heritability values for quantitative traits are due to their sensitivity to the interactions with the environment (Allard, 1999). The analyses of heritability showed that broad and narrow sense heritability were high for all capsaicinoids. Broad and narrow sense heritability for each chemical compound were: capsaicin (H²_b=84% and H²_n=24%), dihydrocapsaicin (H²_b=95% and H²_n=55%),

nordihydrocapsaicin (H²_b=93% and H²_n=77%) and total capsaicinoids (H_b =95% and H²_n =45%, Table 2). According to Singh and Chaudhary (1985), heritability estimates of cultivated crops can be categorized as low heritability (5–10%), medium heritability (11–30%) and high heritability (31–60%). In this study, a high level of broad sense heritability (more than 80%) was found, indicating that the content of all the capsaicinoids are highly heritable with high genetic variance. These results are in agreement with those of Hasanuzzaman et al. (2012) for six genotypes of hot pepper and 11 agronomic traits. The high heritability indicates the existence of additive genes in the expression of traits that could easily be exploited (Bharadwaj et al., 2007). For a trait with low heritability, selection may be quite difficult or virtually impractical due to the masking effect of the environmental interaction on the genotypic effects (Singh, 1990). Therefore, the high heritability values obtained in the present study for all the traits indicates that selection can be applied in the first generations of breeding programs reported by Mishra et al. (1989) and Patel et al. (1997). It is however important to specify that our findings apply for the environment of our study and that additional environment should be tested in the future to confirm our results.

General combining ability effect of the parent

For F₁ hybrid cultivar development, it is necessary to evaluate specific parental combinations. The relative contributions of individual parents to improving specific traits in a population can be estimated by comparing the GCA effects (Lippert, 1975). Zewdie and Bosland (2001) demonstrated that parents with positive and high GCA have the ability to increase the capsaicinoid content in the population. Conversely, parents with negative GCA effects contribute mainly to the reduction of capsaicinoid content.

Table 3: Mean capsaicinoid content (Mean) and general combining ability (GCA) effects of six pepper genotypes for capsaicin (CAP), dihydrocapsaicin (DH), nordihydrocapsaicin (NDH) and total capsaicinoid (CAPT).

Parents	CAP		DH		NDH		CAPT	
	Mean [†]	GCA	Mean	GCA	Mean	GCA	Mean	GCA
P1	0.42 ^g	71 ^{***}	0.29 ^l	-156 ^{***}	0.038 ^{ijk}	-43.16 ^{***}	0.74 ^k	-270 ^{***}
P2	0.67 ^f	60 ^{***}	1.09 ^c	184 ^{***}	0.32 ^a	71.2 ^{***}	2.08 ^c	314 ^{***}
P3	0.45 ^g	125 ^{***}	0.32 ^{hi}	56 ^{***}	0.06 ^{ij}	5.9 ^{ns}	0.85 ^{jk}	187 ^{***}
P4	0.68 ^f	-0 ^{ns¶}	0.64 ^g	-83 ^{***}	0.13 ^{efg}	-20.23 ^{***}	1.46 ^h	-103 ^{***}
P5	0.065 ⁱ	-245 ^{***}	0.039 ^j	-224 ^{***}	0.005 ^k	-56.21 ^{***}	0.10 ^m	-525 ^{***}
P6	0.69 ^f	131 ^{***}	0.88 ^{dc}	224 ^{***}	0.17 ^d	42.5 ^{***}	1.75 ^{efg}	397 ^{***}

(Mean): Means of capsaicinoid content of the parents. Capsaicin, dihydrocapsaicin and total capsaicinoid content are expressed in mg.g-1 dry weight (DW); nordihydrocapsaicin content is expressed in mg.g-1 DW equivalent capsaicin. (†) Means in a column followed by the same letter are not statistically different at the 5% level based on Duncan's new multiple range test.

* Significance of the F statistic at the 0.05 probability level.

** Significance of the F statistic at the 0.01 probability level.

*** Significance of the F statistic at the 0.001 probability level.

‡ns, indicates non-significance at P = 0.05.

Differences in capsaicinoid content and GCA were significant among parents (Table 3). The genitor 'P2' and 'P6' had high mean concentration and positive GCA values for capsaicin, dihydrocapsaicin, nordihydrocapsaicin, and total capsaicinoids. Although, parent 'P3' has low to moderate levels of capsaicinoids but significant positive GCA for all excluding nordihydrocapsaicin. Interestingly, although genitors 'P4' and 'P1' have moderate to high levels of capsaicinoids, they have mostly negative GCA. The 'P5' has low levels of capsaicinoids and negative GCA (Table 3).

Hence, in the present study the parents with high positive GCA, 'P2', 'P6' and 'P3' have the ability to increase the capsaicinoid content in the broader population. Moreover, 'P1' has a capability to increase the capsaicin content. These parents are potential genitors for improving total capsaicinoids content.

By contrast, the parents, 'P4' and 'P5' which have negative GCA effects contribute mainly to a reduction in capsaicinoid content. One of the major goals of this study was to assess GCA for individual capsaicinoid compounds in order to obtain different capsaicinoid profiles. Our results showed, however, that except for 'P1', GCA was either positive or negative for all compounds. This result indicates that it will be difficult to manipulate capsaicinoid profiles to enhance a specific compound using the parents tested. The association of high capsaicinoid contents with high positive GCA in the parents also points to the predominance of additive gene action in capsaicinoids inheritance.

Specific combining ability effect and heterosis of the crosses

In general, to develop a highly pungent commercial F₁ hybrid pepper, the hybrid should have capsaicinoid content greater than that of the highest pungent parent (Zewdie and Bosland, 2001) and thus, high parent heterosis is important in hybrid development. SCA effects and heterosis of the crosses in the F₁ generation are shown in Table 4. Both positive and negative heterosis were observed for the hybrids. The values of high parent heterosis range between -69% to 139%. A number of crosses exhibited significant SCA effect and heterotic effect to increase capsaicin, dihydrocapsaicin, nordihydrocapsaicin and total capsaicinoids. The SCA of total capsaicinoids was mainly due to capsaicin and dihydrocapsaicin in all the crosses, particularly the most pungent crosses 'P2'×'P3', 'P6'×'P3', 'P6'×'P1' and 'P3'×'P1' (Table 4). These results are in agreement with the study of Sanchez-Sanchez et al. (2010) who found that 'Zongolica' was the most pungent parent among the five evaluated, and that one of its crosses ('Zongolica'×'Huatusco') produced 35.7 % more capsaicinoids than its maternal parent 'Zongolica'. This

result was linked to its high GCA and SCA values. Interestingly, a hybrid from moderately capsaicinoid content parents, 'P3'×'P1', had the highest high parent heterosis of 129% for capsaicin, 139% for dihydrocapsaicin, 106% for nordihydrocapsaicin and 131% for total capsaicinoids. The significant difference of parents vs crosses for most of the capsaicinoids (Tables 3 and 4) implies the presence of moderate heterosis. The best high parent heterosis in this study was observed from a hybridization between highly and moderately pungent parents 'P6' x 'P3' and 'P2' x 'P3' as well as between two moderately pungent parents ('P3' x 'P1'). The crosses 'P2'×'P1', 'P4'×'P1' and 'P2'×'P6', which were moderately pungent, had significant negative SCA and heterosis for total capsaicinoids (Table 4). Out of 15 crosses, five displayed significant positive SCA effect and higher degree of heterosis for all capsaicinoid content in green fruits. The best crosses related to capsaicin content and derivatives, were 'P2' x 'P3', 'P6' x 'P3', 'P6'×'P1' and 'P3' x 'P1'. These crosses had higher capsaicin content than the parents and had significant positive SCA with high heterotic effect for total and individual capsaicinoid. In addition, they showed the highest GCA effects for pungency. Such results suggest that additive gene action is important in maximizing the capsaicinoid content of a hybrid.

In contrast, the least pungent crosses, 'P3'×'P5' showed negative SCA values for all capsaicinoids. This cross could be used to develop a fresh market variety with lower pungency. It is however noticeable that SCA and heterosis was either positive or negative for all individual capsaicinoid compounds and that these crosses could either increase or reduce capsaicinoid contents but not likely modify individual capsaicinoid profiles.

IV. CONCLUSION

In this study, genetic crosses for pungency of six Tunisian hot pepper varieties exhibited a high heritability for capsaicinoid content and the predominance of additive gene action in capsaicinoids inheritance. Among the six pepper parents, 'Rouge Long', 'Piment Sesseb' and 'Chaabani' showed that high GCA for capsaicin, dihydrocapsaicin, nordihydrocapsaicin and total capsaicinoids content could be increased by using these parents in a breeding program and may be exploited to improve fruit pungency in the future. The hybrids 'Rouge Long'×'Chaabani' and 'Piment Sesseb'×'Chaabani' had higher capsaicinoid contents than the parents, a high SCA effect and high heterotic effect, which contributed to an additive gene effect. This result shows that these F₁ hybrids expressed higher capsaicinoid content than the parents and this was observed for all individual

compounds. Furthermore, the presence of parents with high combining ability points out at the opportunity for developing F1 hybrids with high pungency.

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Table 4: Mean capsaicinoid content (Mean), specific combining ability (SCA) and high parent heterosis (H) for Capsaicin (CAP), Dihydrocapsaicin (DH), Nordihydrocapsaicin (NDH) and total Capsaicinoid (CAPT) of the hybrids

Hybrids	CAP			DH			NDH			CAPT		
	Mean† (mg.g ⁻¹)	SCA	H (%)	Mean (mg.g ⁻¹)	SCA	H (%)	Mean (mg.g ⁻¹)	SCA	H (%)	Mean (mg.g ⁻¹)	SCA	H (%)
P2xP6	0.48 ^g	-388 ^{***}	-69	0.62 ^g	-430 ^{***}	-43	0.17 ^{de}	-74 ^{***}	-47	1.26 ⁱ	-892 ^{***}	-39
P2xP4	0.68 ^f	-55 [*]	-5	0.68 ^g	-56 ^{ns}	-37	0.16 ^{de}	-16 ^{ns}	-50	1.52 ^{hi}	-128 [*]	-27
P2xP3	1.32 ^b	461 ^{***}	97	1.24 ^b	361 ^{***}	14	0.32 ^a	116 ^{***}	0.5	2.88 ^b	937 ^{***}	38
P2xP1	0.46 ^g	-204 ^{***}	-31	0.38 ^{hi}	-291 ^{***}	-65	0.06 ^{hi}	-87 ^{***}	-81	0.91 ^{jk}	-582 ^{***}	-56
P2xP5	0.93 ^{de}	313 ^{***}	38	0.85 ^{def}	333 ^{***}	-22	0.10 ^{gh}	9 ^{ns}	-69	1.88 ^{de}	655 ^{***}	-9
P6xP4	0.96 ^d	150 ^{***}	39	0.63 ^g	-154 ^{***}	-28	0.11 ^g	-37.5 ^{***}	-35	1.69 ^{fg}	-41 ^{nsq}	-3
P6xP3	1.44 ^a	508 ^{***}	108	1.51 ^a	593 ^{***}	71	0.24 ^b	70 ^{***}	39	3.20 ^a	1171 ^{***}	83
P6xP1	0.88 ^{de}	140 ^{***}	27	0.80 ^{ef}	90 ^{**}	-9	0.15 ^{def}	23 [*]	-12	1.82 ^{def}	253 ^{***}	4
P6xP5	0.67 ^f	-154 ^{***}	-3	0.93 ^d	96 ^{**}	6	0.21 ^c	59 ^{***}	22	1.81 ^{def}	1 ^{ns}	3
P4xP3	0.88 ^c	64 ^{**}	28	0.63 ^g	15 ^{ns}	-2	0.10 ^g	-9.5 ^{ns}	-24	1.70 ^{gh}	70 ^{ns}	16
P4xP1	0.46 ^g	-144 ^{***}	-32	0.30 ⁱ	-105 ^{***}	-53	0.06 ^{ij}	-6 ^{ns}	-54	0.81 ^{jk}	-255 ^{***}	-44
P4xP5	0.41 ^g	-21 ^{nsq}	-39	0.30 ⁱ	132 ^{***}	-53	0.03 ^{jk}	23 [*]	-55	0.73 ^k	134 [*]	-49
P3xP1	1.04 ^c	311 ^{***}	129	0.77 ^f	236 ^{***}	139	0.12 ^{fg}	34 ^{**}	106	1.94 ^{cd}	581 ^{***}	131
P3xP5	0.16 ^h	-873 ^{***}	64	0.12 ^j	-777 ^{***}	-63	0.02 ^k	-132 ^{***}	-66	0.30 ^l	-1783 ^{***}	64
P1xP5	0.49 ^g	15 ^{ns}	17	0.41 ^h	110 ^{***}	42	0.07 ^{hi}	39 ^{***}	85	0.97 ^j	163 ^{**}	30

(Mean): Means of capsaicinoid content of the hybrids F1. H: High parent heterosis = $\{[(F1 - HP)/HP]*100\}$; where HP= high parent mean.

(†) Means in a column followed by the same letter are not statistically different at 5% level according to Duncan's new multiple range test.

* Significance of the F statistic at the 0.05 probability level.

** Significance of the F statistic at the 0.01 probability level.

*** Significance of the F statistic at the 0.001 probability level, (overall comparison of parents and hybrids).

¶ns, indicates non-significance at P = 0.05.