Determination of Combinatorial Ability and Heterosis in *Capsicum chinense* Jacq., using Line×Tester Analysis Method

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ABSTRACT
Objective: Know the combinatory ability and heterosis in yield parameters and fruit quality, in nine genotypes of Habanero Chilli (*Capsicum chinense* Jacq.).
Design/methodology/approach: In order to know the combinatory ability and heterosis in yield parameters and fruit quality, were crossed nine genotypes of *Capsicum c hinense* Jacq. (Habanero Chilli) in a Line×Tester (7×2) mating design. The parental lines and combination of crosses were evaluated in a randomized complete block design with three replications at the Yucatan Scientific Research Center during the 2018 and 2019.
Results: The results showed the lines 3 and 7, presented a high and positive general combinatory ability (GCA) for most of the parameters studied, the tester 1 presented a high GCA for yield per plant (YP) with 0.45. The cross L1×T2 presented the highest value of specific combining ability (SCA) for YP with 0.50. The highest heterosis (171.01%) was observed in the L1×T1 cross for capsaicin content (CC). Tester 1 was identified as a promising genotype for breeding *Capsicum chinense* Jacq.
Findings/conclusions: Line 7 and Tester 1 was identified as promising genotypes for crop breeding *Capsicum chinense* Jacq.

Keywords: *Capsicum chinense* Jacq., Line×Tester Analysis, F1 hybrids, crop breeding, yield.

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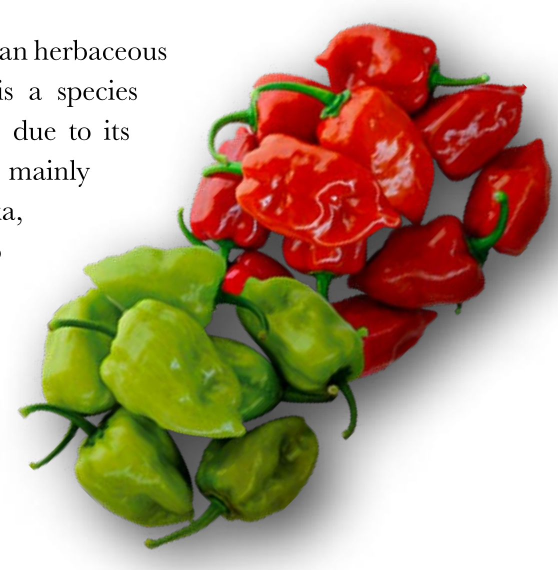
INTRODUCTION

The (*Capsicum chinense* Jacq.) is grown from an herbaceous plant belonging to the genus *Capsicum*, it is a species highly appreciated by international markets, due to its high level of spiciness, flavor and aroma. It is mainly cultivated in Australia, Britain, USA, Sri Lanka, Bangladesh, India, and Mexico, Habanero Chilli the latter country being the one with the greatest genetic diversity of this genus and a growing demand for production (Palma-Orozco *et al.*, 2021), (Cordova *et al.*, 2021).



Plant genetic improvement contributes to improving the sustainability of production systems, through the generation of crops adapted to different environments and consumer demands. However, despite recent scientific discoveries, the need to generate new cultivars persists (Dato *et al.*, 2015)59 accessions belonging to nine different species have been genotyped with a set of ten simple sequence repeats (SSR. The wide genetic diversity of *Capsicum chinense* Jacq. position it as an excellent resource to enter into a genetic improvement program, to obtain better genotypes that increase yield and improve fruit quality.

The main job of plant breeders is to achieve combinations of genes through selection, crossing, mutations and genetic engineering, to obtain the best genotypes, based only the phenotypes (Kempthorne, 1974). By carrying out crosses between various genotypes, we can first obtain F1 hybrids which are the most efficient alternative to satisfy the demands of *Capsicum chinense* Jacq. in the food, pharmaceutical, military, and painting industries, among others (Tag *et al.*, 2014; Martins *et al.*, 2017 and Tyagi *et al.*, 2022). F1 hybrids represent two important advantages, its uniformity and heterosis. However, a great segregation occurs in their progenies, losing the two advantages mentioned and they only maintain these, if they are crossing again with their parents. This is because the F1 hybrids do not reproduce themselves, since their heterozygosity is very high.

The Line×Tester (L×T) analysis is a modified version of the top cross design. The most important advantage of this method is that it allows evaluation between parents with less experimental material compared to other designs. Besides, with the Line×Tester design, the analysis of the level of heterosis and combinatorial ability can be carried out, which are important parameters to define the best parents and introduce them to other genetic improvement programs (Kahriman *et al.*, 2016). Heterosis represents a specific result of the dissimilarity in the constitution of the parents' gametes, which is expressed in greater size, vigor, fecundity manifested by crossed organisms, with respect to inbred organisms (Tuxtla-Andrade *et al.*, 2022).

The present study was carried out with the aim of determining the combinatorial ability and heterosis, as well as knowing the best crosses and their respective parents, in F1 hybrids of Habanero Chilli (*Capsicum chinense* Jacq.), obtained through the Line×Tester genetic improvement design.

MATERIAL AND METHODS

Genetic material

Nine genotypes of *Capsicum chinense* Jacq. were used as parents of which, four are ripe red fruit (T1, L1, L2, L3), two orange (L4, L5), two yellow (L6, L7) and one purple (T2), and were obtained from the CICY (Yucatan Scientific Research Center) gene bank.

Field performance evaluation

The nine parents were crossed in autumn 2018 in a scheme seven lines and two testers (7×2), according to the Line×Tester mating design developed by (Kempthorne, 1974). The process consisted of crossing one parent chosen as tester (P), with the other parents (lines), generating 14 crosses in total (Table 1, Figure 1). For this evaluation, the seeds

Table 1. Design of Line×Tester crosses (7×2) in Habanero Chilli.

Genotypes		
Progenitors	Testers (2)	T1; T2
	Lines (2)	L1; L2; L3; L4; L5; L6; L7
	Crosses (14)	L1×T1; L2×T1: L3×T1; L4×T1; L5×T1; L6×T1; L7×T1; L1×T2; L2×T2; L3×T2; L4×T2; L5×T2; L6×T2; L7×T2

Figure 1. Habanero Chilli genotypes used as testers, in the Line×Tester (7×2) genetic improvement design. A) T1=Tester 1; B) T2=Tester 2.

obtained from the crosses and their respective parents were germinated in commercial substrate Peat moss® (*Spagnum* spp.). The transplant was performed in the summer of 2019, 40 days after sowing in growbags (Pelemix, Guadalajara Jalisco, Mexico) with 1 m a length of, which contains coconut fiber (thick and fine) in a proportion 70:30; the distances between plants were 20 cm and rows were 160 cm, in a greenhouse of the Yucatan Science and Technology Park located in Merida, Yucatan, under hydroponic conditions.

Fruit quality attributes

The fruit quality attributes evaluated in the crosses (F1 hybrids) and their parents were: fruit weight (FW) in grams (g), pericarp thickness (PT), length (FL), width (WF) in centimeters (cm), the parameter yield per plant (YP) in kilograms per plant (kg·plant^{-1}) and the number of fruits per plant (NF) were also evaluated, evaluated according to the Capsicum descriptors (IPGR, 1995). The capsaicin content, was expressed in milligrams per gram of dry weight (DW), was calculated with the method reported by (Muñoz-Ramírez *et al*., 2018) which consisted of taking mature fruits picked randomly per row of each genotype and dried in oven with a circulation of air for 48 h, to later be ground until a fine powder was obtained, 100 mg fruit powder were mixed with 40 mL acetonitrile and maintained in water bath at 80 °C for 4 h with periodic agitation. The extracts were centrifuged (Sigma 2-16kl) at 17,968 **g** for 10 min at 4 °C. The supernatant was collected and filtered through syringe filters with a polytetrafluoroethylene (PTFE) membrane of 0.45 mm (no. 721-1345; Thermo Scientific) in 2 mL vials of amber glass and finally stored at 4 °C until the chromatographic analysis was performed by high-performance liquid chromatography (HPLC) with a brand team mark Agilent series 1200.

Heterosis and combinatorial abilities

Heterosis was estimated based on the average parent, using the following expressions: percentage of heterosis with respect to the average parent;

$$HPM = \left[\left(F1 - PM \right) / PM \right] \times 100$$

where: *HPM*=heterosis mean of the parents, $F1$=Hybrid F1, *PM*=mean of the parents= $\left[\left(P1 + P2 \right) / 2 \right]$ proposed by (Martínez-Martínez *et al.*, 2014). Combinatorial abilities were determined as reported by (Kahriman *et al.*, 2016).

Experimental design and statistical analysis

The arrangement of the treatments (parents and crosses) was carried out based on the complete randomized block design, with four repetitions and eight plants as the experimental unit. The data obtained from the evaluated parameters were subjected to an analysis of variance (ANOVA). The means of the cultivars were compared by a multiple comparison of means by the least significant differences (LSD) test, at a significance level of 5% ($p \leq 0.05$). The statistical analysis was calculated using the SAS program version 9.3 for Windows (Rodrigues *et al.*, 2012).

RESULTS Y DISCUSSION

Field performance evaluation

Table 2 shows the fruit yield and quality parameters of Habanero Chilli genotypes. It can be observed that line 7 presented statistically the highest values in fruit weight, pericarp thickness and fruit width (16.82 g, 2.79 mm and 4.32 cm, respectively). The cross between line 3 and tester T1 had the greatest fruit length (FL) and the greatest number of fruits per plant (NF) with 5.37 cm and 278.1 respectively. The L7×T1 cross produced the highest yield per plant (YP) with 3.38 kg·plant^{-1}, whereas line 3 obtained the highest value in capsaicin content in fruit (CC) with 120.38 mg·g^{-1} DW. In general, the crosses with Tester T1 stood out in most of the parameters evaluated.

Previous studies also reported significant differences for these traits evaluated between F1 crosses; for example, (Tarinta *et al.*, 2023) reported values of NF (50.88 to 75.52) from patters and (44.54 to 108.9) regarding the hybrids, in *Capsicum baccatum*. Similarly, (Figueiredo *et al.*, 2015) in *Capsicum annuum* observed the same effect in CC (2.4 to 731.4 μg/g DW) from patters and (1.7 to 123.6 μg/g DW) regarding the hybrids.

General combinatorial ability

The general combinatorial ability (GCA) indicates the behavior of a particular parent before the crosses in which it participated, evidencing additive genetic effects (Rohini *et al.*, 2017). In the present study, the parents revealed good general combinatorial ability in the performance and quality parameters (Table 3), the values of general combining capacity in the parental lines indicate that the best genotypes to improve each parameter are the following: Line 7 for FW (2.18 g), PT (0.34 mm) and WF (0.40 cm). Line 3 for

Table 2. Fruit yield and quality parameters evaluated in genotypes of Habanero Chilli analyzed in the Line×Tester (7×2) design.

Line	FW (g)	PT (mm)	FL (cm)	WF (cm)	NF	YP (kg·plant^{-1})	CC (mg·g^{-1} DW)
L1	12.73^{fg}	1.93^{k}	4.32^{ghi}	3.14^{fg}	184.11^{ef}	2.34^{gh}	15.70^{s}
L2	9.75^{k}	2.39^{de}	4.02^{jk}	2.81^{j}	239.83^{b}	2.28^{hi}	20.34^{r}
L3	6.75^{n}	1.46^{m}	5.28^{ab}	2.42^{k}	219.82^{cd}	1.48^{n}	120.38^{a}
L4	12.70^{fg}	2.52^{b}	4.81^{ef}	2.94^{hi}	182.42^{ef}	2.32^{h}	28.95^{o}
L5	10.46^{j}	2.27^{fg}	5.03^{cd}	3.01g^{h}	239.22^{b}	2.50^{ef}	29.13^{o}
L6	16.68^{a}	2.34^{ef}	4.48^{g}	3.67^{c}	149.06^{i}	2.49e^{fg}	26.51^{p}
L7	16.82^{a}	2.79^{a}	3.62^{l}	4.32^{a}	155.18^{hi}	2.61^{e}	23.81^{q}
Tester							
T1	14.11^{c}	2.20^{gh}	4.90^{def}	3.01^{gh}	169.85^{fg}	2.39^{fgh}	23.68^{q}
T2	13.53^{de}	1.98^{jk}	4.24^{hi}	2.95^{hi}	104.92^{j}	1.42^{n}	44.54^{fg}
Line×Tester							
L1×T1	13.61^{d}	2.41^{cde}	4.47^{g}	3.21^{ef}	205.84^{d}	2.80^{d}	53.38^{d}
L2×T1	12.05^{h}	2.56^{b}	4.77^{f}	3.14^{f}	232.65^{bc}	2.80^{d}	30.29^{n}
L3×T1	11.56^{i}	1.84^{l}	5.37^{a}	2.98^{hi}	278.10^{a}	3.21^{b}	71.85^{c}
L4×T1	13.41^{de}	2.49b^{c}	5.04^{cd}	3.02^{gh}	227.35^{bc}	2.99^{c}	45.12^{f}
L5×T1	12.71^{fg}	2.39^{de}	5.13^{bc}	3.13^{fg}	233.40^{bc}	2.96^{c}	33.61^{l}
L6×T1	12.88^{fg}	2.41^{cde}	4.89^{def}	3.30^{e}	228.49^{bc}	2.94^{cd}	39.52^{j}
L7×T1	15.12^{b}	2.46^{bcd}	3.91^{k}	3.87^{b}	223.56^{c}	3.38^{a}	48.1^{e}
L1×T2	12.52^{g}	2.16^{hi}	4.84^{ef}	3.21^{ef}	232.41^{bc}	2.91^{cd}	44.02^{g}
L2×T2	9.83^{k}	2.19^{gh}	4.19^{ij}	2.97^{hi}	189.81^{e}	1.87^{lm}	32.30^{m}
L3×T2	9.47^{kl}	1.94^{k}	4.98^{cde}	2.87^{ij}	220.72^{c}	2.09^{jk}	85.05^{b}
L4×T2	9.18^{lm}	2.04^{j}	4.41^{gh}	2.96^{hi}	188.25^{e}	1.73^{m}	40.78^{i}
L5×T2	8.91^{m}	2.06^{j}	4.45^{g}	3.11^{fg}	240.36^{b}	2.14^{ij}	38.81^{k}
L6×T2	12.74^{fg}	2.07^{ij}	4.75^{f}	3.51^{d}	164.94^{gh}	2.10^{jk}	41.70^{h}
L7×T2	13.12^{ef}	2.76^{a}	4.13^{ij}	3.28e	152.40^{hi}	2.00^{kl}	33.05^{l}
Mean	12.20	2.25	4.61	3.17	202.73	2.42	42.22
LSD 0.05	0.45	0.09	0.19	0.12	14.34	0.14	0.60

Values followed by the same letter do not differ significantly (LSD, T≤0.05); FW: fruit weight; PT: pericarp thickness; FL: fruit length; WF: fruit width; YP: yield per plant; NF: number of fruits per plant; CC: capsaicin content, dry weight (DW); LSD=least significant difference.

FL (0.51 cm), NF (33.82) and CC (32.88 mg·g^{-1} DW). Finally, Tester T1 for YP (0.45 kg·plant^{-1}). In a preliminary study reported by (Naves *et al.*, 2022) in *Capsicum annuum* L. the best parents were: LCA625 (P4) for the number of fruits with 8.76, PKM1 (P5) for the shape of the fruit with 0.12 cm, Arka Lohit (P1) and Pusa Jwala (P6) for capsaicin content with 0.04%.

Table 3. General combinatorial ability of the parameters evaluated in Habanero Chilli used in the Line×Tester (7×2) design.

Line	FW (g)	PT (mm)	FL (cm)	WF (cm)	NF	YP (kg·plant^{-1})	CC (mg·g^{-1} DW)
L1	1.13	0.02	−0.01	0.02	3.54	0.29	3.13
L2	−1.00	0.10	−0.19	−0.13	−4.36	−0.23	−14.27
L3	−1.42	−0.38	0.51	−0.26	33.82	0.09	32.88
L4	−0.64	0.00	0.06	−0.19	−7.79	−0.21	−2.62
L5	−1.13	−0.05	0.12	−0.06	21.29	−0.01	−9.36
L6	0.87	−0.03	0.16	0.22	−18.88	−0.04	−4.95
L7	2.18	0.34	−0.65	0.40	−27.62	0.12	−4.82
SE (GCA_l) line	1.32	0.21	0.33	0.22	20.78	0.17	14.86
Tester							
T1	1.11	0.10	0.13	0.05	17.18	0.45	0.43
T2	−1.11	−0.10	−0.13	−0.05	−17.18	−0.45	−0.43
Line×Tester							
SE (GCAt) Tester	0.63	0.054	0.07	0.026	9.91	0.25	0.24

FW: fruit weight; PT: pericarp thickness; FL: fruit length; WF: fruit width; YP: yield per plant; NF: number of fruits per plant; CC: capsaicin content, dry weight (DW); SE: Standard deviation; GCA: General combinatorial ability.

Specific combinatorial ability

Genetic improvement to obtain hybrids in Habanero Chili is little carried out, largely due to a lack of knowledge about heterosis and parent of origin effects in different hybrid combinations (Pech May *et al.*, 2010). The specific combinatorial ability effects (SCA) of the 14 F1 hybrids of *C. chinense* Jacq. are presented in Table 4.

The best crosses for each parameter were the following: L3×T1 for FW (14.08 g), L7×T2 for PT (0.25 mm), L1×T2 for FL (0.32 mm) and NF (30.46), L7×T1 for WF (0.24 cm) and YP (0.24 kg·plant^{-1}), L3×T2 for CC (7.03 mg·g^{-1} DW). The crosses with Tester T2 stood out in specific combinatorial ability for most of the parameters evaluated. [13] in their work with *Capsicum baccatum* report positive effects SCA on the hybrids UENF1629×UENF1732 for YP (0.49 kg·plant^{-1}) UENF1616×UENF1732 for FW (3.08 g) and UENF 1624×UENF 1639 for NF (37.19).

Heterosis

Most of the hybrids were significantly superior to the parents in all the parameters evaluated (Table 5), heterosis was positive in the cross L3×T1 for WF, whereas the cross L7×T1 presented positive values of heterosis for most of the parameters evaluated (Figure 2). On the other hand, the L1×T1 cross presented the highest value of heterosis in CC (171.01%).

Table 4. Specific combinatorial ability of the parameters evaluated in the F1 Habanero Chilli crosses obtained from the Line×Tester (7×2) design.

Crosses (F1)	**FW (g)**	**PT (mm)**	**FL (cm)**	**WF (cm)**	**NF**	**YP (kg·plant^{-1})**	**CC (mg·g^{-1} DW)**
L1×T1	6.74	0.03	−0.32	−0.05	−30.46	−0.50	4.25
L2×T1	4.76	0.09	0.16	0.03	4.24	0.02	−1.43
L3×T1	14.08	−0.15	0.07	0.00	11.51	0.11	−7.03
L4×T1	5.54	0.13	0.19	−0.02	2.37	0.18	1.74
L5×T1	9.50	0.07	0.21	−0.04	−20.66	−0.04	−3.03
L6×T1	9.62	0.08	−0.06	−0.16	14.59	−0.03	−1.51
L7×T1	10.85	−0.25	−0.24	0.24	18.40	0.24	7.00
L1×T2	−2.82	−0.03	0.32	0.05	30.46	0.50	−4.25
L2×T2	−1.19	−0.09	−0.16	−0.03	−4.24	−0.02	1.43
L3×T2	3.37	0.15	−0.07	0.00	−11.51	−0.11	7.03
L4×T2	3.63	−0.13	−0.19	0.02	−2.37	−0.18	−1.74
L5×T2	7.42	−0.07	−0.21	0.04	20.66	0.04	3.03
L6×T2	5.11	−0.08	0.06	0.16	−14.59	0.03	1.51
L7×T2	2.97	0.25	0.24	−0.24	−18.40	−0.24	−7.00
SE (SCA_{lt}) crosses	0.84	0.024	0.03	0.02	3.30	4.83	0.83

FW: fruit weight; PT: pericarp thickness; FL: fruit length; WF: fruit width; YP: yield per plant; NF: number of fruits per plant; CC: capsaicin content, dry weight (DW); SE: Standard deviation; SCA: specific combinatorial ability.

Figure 2. Cross L7×T1 which presented positive values of heterosis, in four of the seven parameters evaluated. A) Crossing L7×T1; B) Progenitors L7.

As we can see, these outstanding crosses are the product of the cross with tester T1, indicating that this is a good tester to improve these parameters. On the other hand, tester T2 only stood out in the LF parameter, with the highest heterosis (13.1%) obtained from the crossing L1×T2. (Zewdie *et al.*, 2001) in their work indicate that the crosses P2×P3, P2×P6 and P3×P4 presented the highest and most positive values of heterosis for yield

Table 5. Percentage for average heterosis in the parameters evaluated in the F1 crosses of Habanero Chilli obtained from the Line×Tester (7×2) design.

Crosses (F1)	FW (g)	PT (mm)	FL (cm)	WF (cm)	NF	YP ($kg \cdot plant^{-1}$)	CC ($mg \cdot g^{-1}$ DW)
L1×T1	1.48	16.59	−3.08	4.26	16.31	18.38	171.01
L2×T1	0.98	1.15	0.69	0.79	1.36	20.03	37.65
L3×T1	10.83	0.17	5.51	9.65	42.74	65.8	−0.25
L4×T1	0.03	5.52	3.71	1.44	29.08	27.03	71.45
L5×T1	3.45	6.65	3.26	3.93	14.11	21.11	27.39
L6×T1	−16.3	6.21	4.32	−1.27	43.29	20.64	57.50
L7×T1	9.46	17.72	−14.47	29.92	62.72	77.08	41.23
L1×T2	−4.63	10.37	13.1	5.44	60.82	54.51	46.12
L2×T2	−15.56	0.11	1.59	3.21	10.11	0.98	−0.42
L3×T2	−6.56	12.5	4.59	6.91	35.94	44.14	3.15
L4×T2	−30	−9.2	−2.6	0.39	31.03	−7.51	11.00
L5×T2	−25.7	−3.31	−3.93	4.39	39.68	9.17	5.42
L6×T2	−15.66	−4.22	9.03	5.86	29.89	7.63	17.37
L7×T2	−13.57	15.84	5.13	−9.67	17.18	−0.87	−2.49

FW: fruit weight; PT: pericarp thickness; FL: fruit length; WF: fruit width; YP: yield per plant; NF: number of fruits per plant; CC: capsaicin content, dry weight (DW).

(84.5, 46.7 y 44.5 % respectively) the *Capsicum annuum* L. A similar result was also observed in the work of (Martínez-Martínez *et al.*, 2014) who determined a positive heterosis with to the average parent in number and weight of fruits per plant variables associated with yield, this in *Capsicum annuum* L. Regarding capsaicin content, (Zewdie *et al.*, 2001) observed heterosis in the capsaicin content in *Capsicum pubenses* hybrids, which means that F1 hybrids can be used to increase the capsaicin content. (Naves *et al.*, 2022) in their study on heterosis for capsacinoid accumulation in hybrids obtained from the genus *Capsicum*, found considerable heterotic effects specifically for capsaicinoid accumulation in the fruit placenta of hybrids, including those derived from non-spicy parents.

CONCLUSIONS

In this study, the parameters fruit weight, pericarp thickness and fruit width (L7), as well as number of fruits per plant, fruit length and capsaicin content (L3), crossed with the best performance of the evaluator (T1), showed the best general combinatorial capacity. The L7×T1 cross showed the best specific combinatorial ability, as well as the greatest number of positive heterosis values, standing out as a promising F1 hybrid, to be mass produced, just as its parents are excellent material to venture into a *Capsicum chinense* Jacq. genetic improvement program.

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