

Article

# Line × Tester Analysis for Morphological and Fruit Biochemical Traits in Eggplant (*Solanum melongena* L.) Using Wild Relatives as Testers

# **Prashant Kaushik**

Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, 46022 Valencia, Spain; prakau@doctor.upv.es; Tel.: +34-963-877-000

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**Abstract:** Wild relatives of eggplant are commonly exploited for eggplant improvement, but the genetic improvement relies on the information of the genetic basis of inheritance of traits. In this study, two eggplant lines, one with oriental and another with occidental cytoplasm, were crossed with four testers representing three wild species, namely, *Solanum insanum, S.anguivi*, and *S. lichtensteinii*. The Line × Tester cross produced a total of eight interspecific hybrids. Parents and their hybrids were evaluated for 3 biochemical, 12 morphological, and 8 Tomato Analyzer-based descriptors. A significant amount of variation was noticed for all 23 traits studied. The higher values for the specific combining ability (SCA) component were determined as compared to the general combining ability (GCA) component. The testers were more significant for most of the traits than the cultivated varieties. Positive heterosis was determined for the 12 characteristics and negative heterosis for the 11 attributes. Overall, *S.anguivi*, and *S. lichtensteinii* were better for the biochemical traits' improvement, whereas *S. insanum* was a better tester for the morphological traits.

Keywords: eggplant; wild relatives; combining ability; Line × Tester analysis; heterosis

# 1. Introduction

The global demand for vegetables is increasing, and this trend is expected to continue in the future [1]. Vegetables, being well adapted to crop rotation, rich in nutrient and minerals, and also highly diverse by nature, can make an effective contribution to address the challenges of food security [2]. Eggplant (*Solanum melongena* L.; Solanaceae) is a highly diverse vegetable with a large array of phenotypically variable local varieties. Several studies show that eggplant can be hybridized with many wild related species, opening the way for introgression breeding by using wild relatives as donors of variation [3,4]. Although the center of origin of eggplant is the Indo–Chinese region [5], the greatest diversity in its wild relatives is found in Africa [6].

Undoubtedly, crop wild relatives are important reservoirs of useful genes and underexploited variation [7]. Wild relatives of eggplant are a source of variation for important traits, such as pest and disease resistance, drought tolerance, and for some quality traits, like a high content in bioactive phenolic acids [8]. Although eggplant is one of the vegetables with the highest concentrations in phenolic acids [9], wild relatives can contribute to a further dramatic increase in these bioactive compounds highly beneficial for human health. Most of the phenolic acids content (usually above 90%) in the eggplant flesh correspond to chlorogenic acid, while in the wild species other phenolic acids such as caffeic acid conjugates may also be present in significant proportions [10,11]. However, most eggplant wild relatives are prickly and generally produce small fruits, which are undesirable traits [12,13]. The eggplant fruit ideotype is variable depending on the final market niche and is based on several morphological and biochemical traits [14]. However, in general, a high content in



phenolic acids seems desirable due to their antioxidant activity and their properties in preventing several diseases [15].

Information on the inheritance of important traits and their gene action is essential to proceed with an efficient genetic improvement of plants. There are several mating designs for obtaining such information, and among these, the Line × Tester (L × T) mating design introduced by Kempthorne [16] allows gaining better insight on the performance of lines and testers in a series of cross combinations. In this design, the line is the female parent which in addition to contributing 50% of the nuclear genes has a cytoplasmic effect on the hybrid, while the tester is the male parent in the cross [17,18]. For lines, the information regarding cytoplasmic inheritance is obtained [19].

The Line × Tester design provides an estimation of the general (GCA) and specific (SCA) combining abilities. The GCA is the estimate of the average performance of a line in a series of cross combinations, and SCA is the performance of a specific cross of better or worse than expected GCA. The GCA and SCA estimates are important to understand the genetic architecture of quantitative traits, and therefore are of great relevance to the establishment of efficient breeding programs [20]. In this way, the usefulness of wild species and cultivated varieties in a breeding program largely depends on the combining ability estimates for traits of interest. Also, the heterotic performance of cross combinations depends on the combining ability of the parents involved in the cross [21,22]. In eggplant, the earliest reports of the estimation of combining ability effects date back to the late 1940s [23]. However, few studies have dealt with the estimations of CGA and SCA in crosses with wild relatives [24]. In a recent study using a diallel cross in which one accession of the wild eggplant relative *S. insanum* was included, we found that GCA and SCA estimates were significant for most of the morphological traits. Also, the wild relative *S. insanum* had low values for GCA fruit-related morphological traits [21].

Heterosis is commonly used to measure the superiority of hybrids with respect to their parents and heterosis estimate of a trait is the result of additive and non-additive gene actions [25,26]. Whereas, heritability is a significant predictor of the selection response for a particular trait in the subsequent generations. In eggplant, the first success in the development of heterotic hybrids for agronomic traits was recorded in the 1890s [23,27]. Thereafter, heterosis breeding has become an important routine in eggplant improvement [27]. Previously, we have evaluated the heterosis for the agronomical and biochemical traits in eggplant, using crosses with wild relatives as well as with cultivated parents [11,13,21]. However, to our knowledge, up to now there are no studies using the L  $\times$  T breeding design in eggplant using wild species as testers. Therefore, the overall objectives with this study were to determine the combining ability, gene action, heterosis, and heritability of important morphological, morphometric, and biochemical traits using four eggplant wild relatives as testers against two eggplant lines, one from the occidental group and another one from the oriental group [28].

## 2. Materials and Methods

#### 2.1. Plant Material and Growing Conditions

Two cultivated eggplant (*S. melongena*) lines, one from the Ivory Coast (MEL3; occidental group), and one from Sri Lanka (MEL4; oriental group) were used as the female parent lines (Table 1). Four accessions of eggplant wild relatives, of which two were from the primary gene pool species *Solanum insanum* (INS1 and INS2), and two from the secondary genepool species *S. anguivi* (ANG1) and *S. lichtensteinii* (LIC2) were used as male parents (testers) (Figure 1). The mating of lines by testers produced eight interspecific hybrids (Table 1). The lines, testers, and the L × T interspecific crosses were grown in an experimental field at the Universitat Politècnica de València (Valencia, Spain; GPS coordinates of the plot:  $39^{\circ}28'55''$  N,  $0^{\circ}22'11''$  W; altitude 7 m a.s.l.). Five plants (each plant was a replication) of each of the lines, testers, and L × T interspecific hybrids were distributed in a randomized complete block design in an open field plot. The plant-to-plant and row-to-row spacings were 1.2 m and 1.0 m, respectively. The plants were irrigated with a drip irrigation system and fertilized using 80 g plant<sup>-1</sup> of a 10 N-2.2 P-24.9 K plus micronutrients fertilizer (Hakaphos Naranja; Compo

Agricultura, Barcelona, Spain), which was distributed throughout the cultivation period with the drip irrigation system.

Species	Accession	Germplasm	Country of	Interspecific Hybrids				
	Code Collection Code		Origin	With MEL3	With MEL4			
	Cultiva	ted Eggplant						
S. melongena	MEL3	BBS-175	Ivory Coast					
_	MEL4	7145	Sri Lanka					
	Wild primar	ry genepool (GP1)						
S. insanum	INS1	SLKINS-1	Sri Lanka	$MEL3 \times INS1$	$MEL4 \times INS1$			
	INS2	SLKINS-1	Sri Lanka	$MEL3 \times INS2$	$\text{MEL4}\times\text{INS2}$			
	Wild seconda	ry genepool (GP2)						
S. anguivi	ANG1	BBS119	Ivory Coast	MEL3 $\times$ ANG1	$MEL4 \times ANG1$			
S. lichtensteinii	LIC2	MM677	Iran	$MEL3 \times LIC2$	$MEL4 \times LIC2$			

**Table 1.** Accessions of cultivated eggplant (lines) and wild relatives (testers) used for the line by tester analysis.



Figure 1. Fruits of six eggplant accessions used in the Line × Tester study.

# 2.2. Characterisation and Data Analysis

Line and tester parents and their resultant interspecific hybrids were characterized for the 12 conventional morphological descriptors as defined by the EGGNET and IBPGR [29,30]. Five measurements were recorded in each replication except for plant height and stem diameter.

Five plants per replicate were collected at the commercial ripe stage for the fruit morphometric and biochemical characterization. Eight fruit morphometric traits were also scored using the popular Tomato Analyzer version 4 software [31]. For the fruit morphometric analysis, the fruits were cut open longitudinally and scanned with the help of an HP Scanjet G4010 photo scanner (Hewlett Packard, Palo Alto, CA, USA) at 300 dpi. A brief list of different descriptors used for the characterization of parents and their hybrids is presented in Table 2.

Traits/Descriptors	Scale
Biochemical Traits	
Phenolics	mg/g
CGA	mg/g
Dry Matter	%
Morphological Traits	
Fruit Pedicel Length	mm
Fruit Pedicel Diameter	mm
Fruit Weight	g
Stem Diameter	mm
Plant Height	cm
Leaf blade length	cm
Leaf Blade Lobing	1 = Very weak (none); 9 = Very Strong
Leaf Blade Width	cm
Number of Flower Prickles	0 = None; 9 = Very many (>20)
Number of Flowers Per Inflorescence	-
Corolla Color	1 = Greenish white; 9 = Bluish violet
Corolla Diameter	mm
Tomato Analyzer-Based Descriptors	
Perimeter	cm
Area	cm <sup>2</sup>
Height Mid-Width	cm
Maximum Height	cm
Curved Height	cm
Fruit Shape Index External I	The ratio of maximum height to maximum width.
Fruit Shape Index External II	The ratio of height mid-width to width mid-height.
Distal Fruit Blockiness	The ratio of the width at the lower blockiness position to width mid-height.

**Table 2.** List of different biochemical, morphological, and Tomato Analyzer-based traits/descriptors used for the characterization.

Snap-frozen tissues of fruit flesh samples were lyophilized and grounded to the fine powder consistency. This fine powder was used for the estimation of three biochemical traits (dry matter, total phenolics, and chlorogenic acid content). Dry matter was estimated as the change of weight in the fresh sample before and after lyophilization based on the formula  $100 \times (dry weight/fresh weight)$  and expressed as dry matter percentage. The total phenolics were estimated using the Folin–Ciocalteu method defined elsewhere [11,32]. The chlorogenic acid (CGA) content was determined with the help of high-performance liquid chromatography (HPLC) system using a standard solution of CGA as a control. The analysis was performed on to a 1220 Infinity LC System (Agilent Technologies, Santa

Clara, CA, USA). The results were computed by the OpenLAB CDS ChemStation Edition software package (Agilent Technologies) following the manufacturer's instructions.

Average values for lines, testers, and L × T hybrids are provided in Table S1. The estimation of general combining ability (GCA) and the specific combining ability (SCA) including the variance and its contribution effects were performed based on the traditional linear model of L × T analyses [16]. The heterosis was estimated over the mid-parent values (H; %) hybrids using the formula as H = 100 × ((F1 – MP)/MP), where  $F_1$  = hybrid mean, and MP = mean of the parents. All these calculations were performed with the help of the software package AGD-R version 5.0 [33].

## 3. Results

#### 3.1. Analysis of Variance for Line, Tester, and L × T Effects and GCA and SCA Estimates

The average values of parents and their hybrids were different, and a wide range of variation was present for all of the traits studied Table S1. The analysis of variance for combining the abilities of the 23 descriptors studied in an  $L \times T (2 \times 4)$  design is presented in Table 3. The mean squares due to treatments were highly significant for all the traits (Table 3). But, the mean squares due to the lines (female) were significant for only nine traits out of the total twenty-three. The lines were significant for four morphological-based descriptors and five Tomato Analyzer-based descriptors, and were not significant for any of the biochemical traits studied (Table 3). Whereas, testers were significant for the fruit phenolics, including thirteen other traits, out of which ten were morphological traits, and two were measured with the Tomato Analyzer (Table 3). Nineteen traits out of the total twenty three traits were determined to be significant for the fourteen traits composed of morphological and Tomato Analyzer-based descriptors, thereby showing the heterotic effect for more than half of the studied traits (Table 3). Overall, the SCA effects were several times higher than the GCA effects for every trait excluding the morphological trait number of flowers per inflorescence (Table 3). Thus, leading to an overall lower estimate of GCA/SCA ratio (<0.5) (Table 3).

Source of Variation	Replicat	es Treatments	Parents	Lines	Testers	Lines vs Testers	Parents vs Hybrids	Hybrids	Lines	Testers	Lines X Testers	Error	s <sup>2</sup> GCA	s <sup>2</sup> SCA	GCA/SCA
d.f	2	13	5	1	3	1	1	7	1	3	3	26			
Phenolics	10.86	46.95 ***	54.31 ***	4.58	50.43***	11 5.67	15.15	46.25 ***	1.69	0.96	106.40 ***	4.84	0.07	53.20	0.001
CGA	0.06	1.56 ***	0.59	0.07	0.27	2.11 **	0.97	2.35 ***	1.15	1.29	3.81 ***	0.24	0.05	1.91	0.025
Dry Matter	0.04	46.35 ***	7.21	19.3	3.7	5.67	0.09	80.92 ***	99.35	8.09	147.60 ***	5.01	4.14	73.80	0.056
Fruit Pedicel Length	2.34	488.86 ***	477.05 ***	60.17 **	118.64 ***	1969.14 ***	5.98	566.28 ***	598	3.5	1118.50 ***	5.89	24.91	559.24	0.045
Fruit Pedicel Diameter	0.09	28.82 ***	25.81 ***	1.5	14.81 ***	83.11 ***	1.04	34.94 ***	23.21	16.96	56.83 ***	0.39	0.96	28.41	0.034
Fruit Weight	152.26	29,905.50 ***	20,384.54 ***	411.35	154.91	10,1046.60 ***	16,454.45 ***	38,627.77 ***	56,326.5	15,950.7	55,405.25 ***	558.62	2346.93	27,702.63	0.085
Stem Diameter	5.22	51.15 ***	18.55	10.67	13.46	41.71 *	78.77 **	70.49 ***	93.02	61.89	71.57 **	9.82	3.87	35.78	0.108
Plant Height	16.67	1879.98 ***	1715.83 ***	32.67	948.75 ***	5700.25 ***	6396.44 ***	1352.02 ***	3762.52	742.32	1158.23 ***	83.22	156.77	579.11	0.271
Leaf Blade Length	0.23	77.67 ***	26.58 ***	21.09 ***	37.24 ***	0.07	157.58 ***	102.76 ***	13.28	10.37	224.97 ***	1.47	0.55	112.48	0.005
Leaf Blade Lobing	0.01	5.27 ***	3.20 ***	6.00 ***	3.00 ***	1.00 ***	4.57 ***	6.86 ***	6	12	2.00 ***	0.01	0.25	1.01	0.248
Leaf Blade Width	0.56	48.60 ***	21.45 ***	0.98	35.36 ***	0.18	140.26 ***	54.91 ***	13.37	6.93	116.74 ***	0.65	0.55	58.37	0.009
Number of Flower Prickles	0.87	16.17 ***	11.30 ***	1.5	17.00 ***	4.00 *	27.86 ***	17.99 ***	21.09	21.84	13.10 ***	0.88	0.87	6.54	0.133
Number of Flowers per Infloresence	0.39	22.41 ***	23.68 ***	0.02	14.43 ***	75.10 ***	84.26 ***	12.67 ***	50.85 **	12.03*	0.57	0.34	2.11	0.28	7.536
Corolla Color	0.07	8.40 ***	11.60 ***	6.00 ***	16.00 ***	4.00 ***	0.45	7.23 ***	0.38	10.38	6.38 ***	0.07	0.02	3.18	0.005
Corolla Diameter	15.98	328.41 ***	96.97 ***	7.48	81.68 ***	232.31 ***	301.63 ***	497.56 ***	106.26	57.77	1067.77 ***	6	4.42	533.88	0.008
Perimeter	0.45	277.04 ***	335.48 ***	5.66	2.33	1664.76 ***	209.84 ***	244.90 ***	43.63	84.2	472.70 ***	6.75	1.81	236.35	0.008
Area	23.02	877.33 ***	1166.98 ***	15.06	0.93	5817.05 ***	328.38 *	748.87 ***	150.87	229.38	1467.70 ***	56.89	6.28	733.84	0.009
Height Mid-Width	0.12	30.77 ***	37.84 ***	4.24 *	0.19	184.40 ***	17.70 ***	27.60 **	1.01	4.52	59.52 ***	0.8	0.04	29.75	0.001
Maximum Height	0.11	31.76 ***	38.96 ***	3.60 *	0.2	190.61 ***	18.42 ***	28.52 ***	1.16	4.92	61.25 ***	0.8	0.05	30.62	0.002
Curved Height	0.05	31.23 ***	36.90 ***	3.39	0.19	180.55 ***	19.47 ***	28.88 ***	1.66	5.19	61.63 ***	0.83	0.07	30.81	0.002
Fruit Shape Index External I	0.01	0.30 ***	0.14 ***	0.19 ***	0.05 *	0.36 ***	0.01	0.46 ***	0.01	0.24	0.83 ***	0.01	0.01	0.41	0.024
Fruit Shape Index External II	0.01	0.34 ***	0.16 ***	0.27 ***	0.05 *	0.39 ***	0.01	0.52 ***	0	0.28	0.93 ***	0.01	0.01	0.46	0.022
Distal Fruit Blockiness	0.01	0.02 ***	0.01 ***	0.01 **	0.01	0.03 ***	0	0.03 ***	0.02	0.01	0.05 ***	0.01	0.01	0.02	0.500

Table 3. Analysis of variance for general combining ability (GCA) and specific combining ability (SCA) for the descriptors studied for the characterization.

\*\*\*, \*\*, and \* indicate significance at p < 0.001, p < 0.01, or p < 0.05, respectively.

The proportional contributions to the total variance of hybrids by lines, testers, and their interaction as interspecific hybrids  $(L \times T)$  is provided in Table 4. The interspecific hybrids showed the most significant contributions in the expression of the traits, thereafter the testers and lines, as there were the higher values of SCA variance for the traits (Table 4). Except for the traits: leaf blade lobbing, the number of flower prickles and the number of flowers per inflorescence; the interspecific hybrids  $(L \times T)$  contributed the largest portion of the variance. (Table 4). The contribution of  $L \times T$  was above 75% for the thirteen traits out of a total twenty-three, and the traits which received more than 95% of the contribution were phenolics, leaf blade length, corolla diameter, height mid-width, maximum height, and curved height, respectively (Table 4). Subsequently, testers contributed more than the lines for all the traits, except for the fruit-related traits, i.e., fruit weight, fruit length, and fruit diameter (Table 4).

Table 4. Contribution of lines, testers, and their cross	as $(L \times T)$ in the expression of characters studies
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Traits	Lines	Testers	L × T
Phenolics	0.52	0.89	98.59
CGA	7.02	23.45	69.54
Dry Matter	17.54	4.29	78.17
Fruit Pedicel Length	15.09	0.26	84.65
Fruit Pedicel Diameter	9.49	20.8	69.71
Fruit Weight	20.83	17.7	61.47
Stem Diameter	18.85	37.63	43.52
Plant Height	39.76	23.53	36.71
Leaf Blade Length	1.85	4.32	93.83
Leaf Blade Lobbing	12.5	75.02	12.5
Leaf Blade Width	3.48	5.41	91.11
Number of Flower Prickles	16.75	52.05	31.2
Number of Flowers Per Inflorescence	57.36	40.71	1.94
Corolla Color	0.74	61.48	37.78
Corolla Diameter	3.05	4.98	91.97
Perimeter	2.54	14.73	82.72
Area	2.88	13.13	83.99
Height Mid-Width	0.52	7.02	92.46
Maximum Height	0.58	7.39	92.03
Curved Height	0.82	7.71	91.47
Fruit Shape Index External I	0.18	22.41	77.4
Fruit Shape Index External II	0.02	23.00	76.98
Distal Fruit Blockiness	8.26	15.84	75.9

## 3.3. GCA and SCA

The estimates obtained for the GCA effects are provided in Table 5. In the case of biochemical traits, the GCA values of parents were non-significant except for the dry matter content for which MEL4 showed the highest significant GCA value. Whereas, among the four testers ANG1 was determined to be most notable for the CGA content (Table 5). Interestingly, both of the lines, i.e., MEL3 and MEL4 were determined to be reverse complementary to each other for all the twenty-three traits studied (Table 5). For the twelve morphological descriptors among both of the parents, MEL3 was determined to be highly significant for fruit pedicel length and the occidental accession MEL4 was determined to be positively highly significant for the number of flowers per inflorescence (Table 5). Likewise, among testers, INS2 was the best general combiner for the fruit pedicel diameter and fruit weight (Table 5). In the case of the Tomato Analyzer-based descriptors, LIC2 was the best general combiner among the parents for perimeter, height mid-width, maximum height, and curved height, whereas INS1 was for both of the fruit-shaped index external I and II (Table 5).

	Li	nes				
Traits/Characters	MEL3	MEL4	INS1	INS2	ANG1	LIC2
Phenolics	-0.27	0.27	-0.28	0.24	0.43 *	-0.4
CGA	0.22	-0.22	-0.44	-0.23	0.63 *	0.05
Dry Matter	-2.03 **	2.03 **	-0.46	1.45	0.3	-1.29
Fruit Pedicel Length	4.99 ***	-4.99 ***	-0.53	0.71	-0.78	0.6
Fruit Pedicel Diameter	0.98 ***	-0.98 ***	-0.82 ***	2.39 ***	-1.45 ***	-0.12
Fruit Weight	48.45 ***	-48.45 ***	26.96 *	57.12 ***	-57.06 ***	-27.02 *
Stem Diameter	-1.97	1.97	4.24 *	-3.14 *	-1.68	0.57
Plant Height	-12.52 ***	12.52 ***	16.6 ***	-6.9 *	-4.23	-5.48
Leaf Blade Length	-0.74	0.74	0.84	0.79	-1.94 **	0.31
Leaf Blade Lobing	-0.50 ***	0.50 ***	-2.00 ***	0.01 ***	1.00 ***	1.00 ***
Leaf Blade Width	-0.75 *	0.75 *	-0.46	0.22	-1.14 **	1.38 **
Number of Flower Prickles	-0.94 *	0.94 *	-2.81 ***	1.19 *	1.19 *	0.44
Number of Flowers Per Inflorescence	-1.46 ***	1.46 ***	0.47	1.38 ***	-1.97 ***	0.12
Corolla Color	0.13	-0.13	-1.13 ***	-1.13 ***	0.88 ***	1.38 ***
Corolla Diameter	2.1 *	-2.1 *	-3.54 *	0.4	-0.8	3.95 **
Perimeter	1.35	-1.35	-2.82	2.97 *	-3.63 *	3.48 *
Area	2.51	-2.51	-3.44	5.84	-6.94	4.53
Height Mid-Width	0.21	-0.21	-0.32	0.03	-0.88	1.17 *
Maximum Height	0.22	-0.22	-0.35	0.04	-0.91	1.22 *
Curved Height	0.26	-0.26	-0.36	0.2	-1.02 *	1.18 *
Fruit Shape Index External I	-0.02	0.02	0.16 ***	-0.29 ***	0.06	0.07
Fruit Shape Index External II	-0.01	0.01	0.20 ***	-0.3 ***	0.05	0.05
Distal Fruit Blockiness	0.03	-0.03	0.04	-0.02	-0.05 *	0.04

Table 5. Estimates of the general combining ability (GCA) effect for the descriptors studied.

\*\*\*, \*\*, and \* indicate significance at p < 0.001, p < 0.01, or p < 0.05, respectively.

The SCA variation with respect to the mean is provided in Table 6. Among the biochemical traits, the highest fluctuation for SCA was recorded for the phenolics ( $\pm 49\%$ ). For morphological traits, the lowest fluctuations, i.e., below  $\pm 12\%$ , were determined for the traits, plant height, leaf blade lobbing, and the number of flowers per inflorescence. The highest fluctuations, i.e., above 85%, were observed for the fruit weight. Likewise, for the Tomato Analyzer-based descriptors, the lowest range was for distal fruit blockiness ( $\pm 17.57\%$ ). In the case of the remaining Tomato Analyzer-based descriptors area, height mid/width, maximum height, and curved height SCA values ranged above  $\pm 70\%$ . Overall, eight out of the total twenty-three traits ranged between -40% to 50% for SCA values (Table 6).

**Table 6.** Range of specific combining ability estimates with respect to mean and mid-parent heterosis for the traits.

Traits	Minimum	Maximum	Mid-Parent Heterosis
Phenolics	-49.35	49.35	-8.96
CGA	-44.73	44.73	-10.52
Dry Matter	-34.69	34.69	0.67
Fruit Pedicel Length	-45.08	45.08	-2.63
Fruit Pedicel Diameter	-43.38	43.38	5.34
Fruit Weight	-86.33	86.33	65.59
Stem Diameter	-17.02	17.02	-10.72
Plant Height	-9.77	9.77	-17.30
Leaf Blade Length	-40.49	40.49	-20.43
Leaf Blade Lobing	-10.00	10	-11.76
Leaf Blade Width	-43.55	43.55	-25.24
Number of Flower Prickles	-73.36	73.36	141.07
Number of Flowers Per Inflorescence	-11.52	11.52	-41.90
Corolla Color	-21.97	21.97	-3.91
Corolla Diameter	-55.35	55.35	-15.34
Perimeter	-60.53	60.53	40.99
Area	-70.71	70.71	40.39
Height Mid-Width	-78.14	78.14	37.05
Maximum Height	-77.37	77.37	37.16
Curved Height	-71.51	71.51	36.33
Fruit Shape Index External I	-43.33	43.33	2.71
Fruit Shape Index External II	-47.00	47	2.97
Distal Fruit Blockiness	-17.57	17.57	-1.18

### 3.4. Heterosis

The lowest value for the overall mid-parent heterosis was noticed for the number of flowers per inflorescence (-41.9%), whereas the highest mid-parent heterosis was noticed for the number of flower prickles (141.1%) (Table 6). The negative mid-parent heterosis was determined for the traits phenolics, CGA, stem diameter, plant height, leaf blade length, leaf blade lobbing, leaf blade width, corolla color, corolla diameter, and distal fruit blockiness (Figure 2). In contrast, the positive value for mid-parent heterosis was determined for the dry matter, fruit pedicel diameter, fruit weight, perimeter, area, height mid-width, maximum height, curved height, and fruit shape index externals I and II, respectively (Figure 2). The mid-parent heterosis for the dry matter was less than 1%. Whereas, it was around 3% for the fruit shape index externals I and II. Significantly negative heterosis was determined for all the leaf-based traits, i.e., leaf blade length (-20.4%), leaf blade lobbing (-11.8%), and leaf blade width (-25.2%) (Table 6).



Figure 2. Heterosis over mid-parent values for all the descriptors studies.

## 4. Discussion

The phenotypic selection of parents is still key to the improvement of many vegetables for quantitative traits, especially in resource-limited circumstances [34,35]. The Line  $\times$  Tester, a well-established biometrical genetics-based approach, gives a better estimate and sure prediction of

the important quantitative traits as seen for other solanaceous vegetables including eggplant [36–38]. Any improvement of traits would ultimately depend on the genetic nature and magnitude of gene action [39]. The mean squares due to GCA, SCA, and GCA/SCA ratios points out the magnitude of gene action, and this further aids in developing an appropriate breeding strategy for future breeding programs [20].

In our study, the two lines, one with oriental and another with occidental cytoplasm, were crossed with four testers representing three wild species. This diverse germplasm helped in the precise estimation of the basis of inheritance of 3 biochemical, 12 morphological, and 8 Tomato Analyzer-based descriptors. A significant amount of variation was noticed for all of the 23 traits studied. Overall, larger values for the SCA component compared to GCA were noticed. This may be due to the larger genetic distances, as only wild species were used as the testers [40,41]. The higher SCA values have resulted in low GCA/SCA, pointing out the presence of non-additive effects governing all the traits studied except for the number of flowers per plant [22] Among all the genotypes studied, only the accession of the secondary genepool's wild relative of eggplant S. anguivi was found to be significant for the biochemical traits. The eggplant has a huge diversity in shape based on its local landraces and wild species cultivated in different countries. The popular variety is based on local preferences [37]. The secondary genepool species are the reserve of useful genes for the improvement of present-day varieties, but because of breeding barriers, they are not exploited to their full potential [4,42,43]. Therefore, most of the time, the local germplasm is used extensively which might have resulted in the lower genomic diversity of eggplant, which has further resulted in the yield stagnation and susceptibility to diseases [44]. Similarly, for most of the other trait testers, they were more significant in values than the cultivated lines, although both of the lines had different cytoplasm.

The information on GCA's effects provides a relative picture of which genotypes are important for selection and further exploitation in breeding programs. The positive and negative SCAs and their values are also important for some characteristics, as some need to be more positive than negative. In the case of obtaining precise information regarding the behavior of wild species with less information and less utilization in crop breeding programs, it is one of the best choices. The lowest fluctuation was noticed for the plant height to the maximum fluctuation for fruit weight. Recently, a similar amount of heterosis was noticed in a diallel matting design study and it was found that single nucleotide polymorphism (SNPs) are not the replacement for biometrical study in the case of eggplant [21]. It was revealed that there was positive heterosis for the 12 traits and negative heterosis for the 11 traits. The positive heterosis was determined mostly in the case of all Tomato Analyzer-based descriptors and negative values for most of the biochemical and morphological descriptors. Earlier heterosis is well reported and exploited in eggplant with respect to several traits [27]. Overall, in our study, most of the traits are shown to be governed by non-additive gene actions. Earlier studies reported both additive and non-additive gene actions governing several important traits of eggplant [21,45].

**Supplementary Materials:** The following are available online at http://www.mdpi.com/2073-4395/9/4/185/s1, Table S1: The mean performance of parents and their hybrids for the traits studied.

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