



## Selection of melon genotypes with resistance to *Didymella bryoniae* using a diallel approach

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**ABSTRACT.** The development of melon lines resistant to gummy stem blight (GSB) is an important strategy for decreasing losses caused by this disease. Thus, selecting the best parents for such a goal is essential. We evaluated the general (GCA) and specific (SCA) combining abilities of lines and accessions to guide the selection of the most promising parents to develop GSB-resistant lines. Six genotypes representing two groups (group I - susceptible; group II - resistant) were crossed in a partial diallel mating design. The genotypes were evaluated for disease expression using a rating scale from 0 (resistant) to 4 (susceptible). Estimates of GCA were negative and significant for the accessions PI 420145 and PI 482398, which can be suggested for crosses aimed at obtaining melon lines resistant to GSB. There is a reciprocal effect for the resistance of melon genotypes to *Didymella bryoniae*, especially when PI 482398, PI 420145, and PI 140471 are crossed with JAB-20. Such results indicate that maternal effects may be involved in the resistance to gummy stem blight.

**Keywords:** *Cucumis melo*; *Didymella bryoniae*; partial diallel; reciprocal effect.

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### Introduction

Gummy stem blight (GSB) is one of the most destructive diseases in cucurbits worldwide, especially for melon (Keinath, 2011; Keinath, 2015). In Brazil, GSB is one of the most damaging diseases in melon fields (Gasparotto, Vida, Tessmann, & Alves, 2011; Cardon et al., 2016). Its aetiologic agent is the fungus *Didymella bryoniae*, frequently leading to symptoms such as cankers, with gum exudation on the stem, rods and petioles, eventually leading to plant death. The pathogen is found in crop residues, infested soil and seeds (Gasparotto et al., 2009).

Chemical control has been used to minimize the plant losses; however, even recommended fungicides have shown problems, such as low efficacy and resistance of the pathogen to some chemical groups, including benzimidazoles and thiophanates, pyrimidines-carboxamides, and methoxycarbamates (Keinath, 2015).

To date, there are no melon cultivars on the market with resistance to *D. bryoniae*, due in large part to the difficulty in finding genotypes with stable resistance. To develop resistant cultivars, some basic requirements are knowledge of pathogen variability, reliable sources of resistant genotypes and knowledge of the mechanisms involved in host resistance.

In an allogamous breeding programme, selecting parents capable of producing segregated populations with desired traits is a crucial step, since it is closely related to the success of subsequent steps when developing superior lines.

Diallel crosses in the selection of parents and, in particular, partial diallel crosses are useful when the objective is to combine favourable phenotypes that are found in different groups of parents, as in the case of productive and susceptible lines and wild and resistant accessions, where the combinations within each group are not of interest. The partial diallels have been used for this purpose in other vegetable crops, such as zucchini (Nogueira et al., 2011) and tomato (Pádua et al., 2010; Andrade et al., 2014).

One of the most common diallel analysis methods is that proposed by Griffing (1956), which provides information on the general combining ability (GCA) and the specific combining ability (SCA) of parents through artificial crosses. It is widely used in several species, supporting the understanding of genetic effects involved in the determination of characters, as well as allowing the identification of hybrid

combinations of agronomic interest (Engelsing, Rozzetto, Coimbra, Zanin, & Guidolin, 2011).

The School of Agrarian and Veterinarian Sciences of the São Paulo State University (UNESP-FCAV) has developed high-yield melon lines (Vargas et al., 2010). However, those lines are susceptible to *D. bryoniae*, requiring the incorporation of genetic resistance. Thus, the success of a breeding programme in obtaining more resistant genotypes depends directly on selecting the best parents to direct the introgression of such a trait into promising lines.

Thus, crossings of parents with contrasting traits are essential for evaluating and selecting the best strategy for the development of resistant lines. Therefore, the objective of this work was to determine the values of the general and specific combining abilities in six melon genotypes for resistance to *D. bryoniae*.

## Material and methods

### Parents and partial diallel cross

Six genotypes were separated into two groups (Group I - *reticulatus* and Group II - wild *melo*). Each genotype from Group I was crossed with all genotypes of Group II, resulting in eight F<sub>1</sub> hybrids and eight reciprocal hybrids, as shown in Table 1.

**Table 1.** Parents, F<sub>1</sub>s and their reciprocals of melon genotypes resistant (Group I) and susceptible (Group II) to *Didymella bryoniae*.

Genitors	Crosses	
	F <sub>1</sub> s	Reciprocals
Group I	1 × 3	3 × 1
1. JAB-11	1 × 4	4 × 1
2. JAB-20	1 × 5	5 × 1
	1 × 6	6 × 1
Group II		
3. PI 140471	2 × 3	3 × 2
4. PI 157082	2 × 4	4 × 2
5. PI 420145	2 × 5	5 × 2
6. PI 482398	2 × 6	6 × 2

The hybrid seeds were obtained from August to December of 2014. Ten plants of each line and of each plant introduction (PI) were cultivated to produce hybrids and reciprocals. The emasculation of the androgenic flowers was performed manually when they were in the floral bud stage, followed by covering emasculated buds with paper bags to avoid pollen contamination; such procedures were performed in the early hours of the day when temperatures were mild.

For pollination, the male flowers were previously protected to avoid contamination, and pollen collection was performed in the afternoon, when pollen release is most efficient. After pollination, buds were once again protected with a paper bag, and crosses were identified by a tag. After fruit set, the protective bag was removed.

After complete maturation, the fruits were harvested, and the seeds were manually removed. Afterwards, seeds were washed in running water and dried at room temperature on paper towels. Then, seeds were acclimated in labelled paper bags and stored in a cold room at a temperature of 10°C.

### Experimental design

The experiment was carried out from March to April 2015 in the School of Agrarian and Veterinarian Sciences (UNESP-FCAV) located at the geographic coordinates latitude 21°15'22" S and longitude 48°18'58" W and an altitude of 595 metres.

For this experiment, we used six parents, crossed in partial diallel, as described in Table 1. All experiments were evaluated in trays according to the method proposed by Santos et al. (2013). The trays were filled with a mixture of subsoil, cattle manure and commercial substrate (Bioplant Agrícola Ltda, Nova Ponte, Minas Gerais State, Brazil) in a 2:1:1 ratio. The mixture was sieved and autoclaved prior to sowing at 120°C and 101.3 kPa for 45 min. Two seeds of each genotype were sown per tray cell (128 wells), and subsequently, thinning of the plants was performed, taking into account their uniform distribution relative to the other plants of the same genotype.

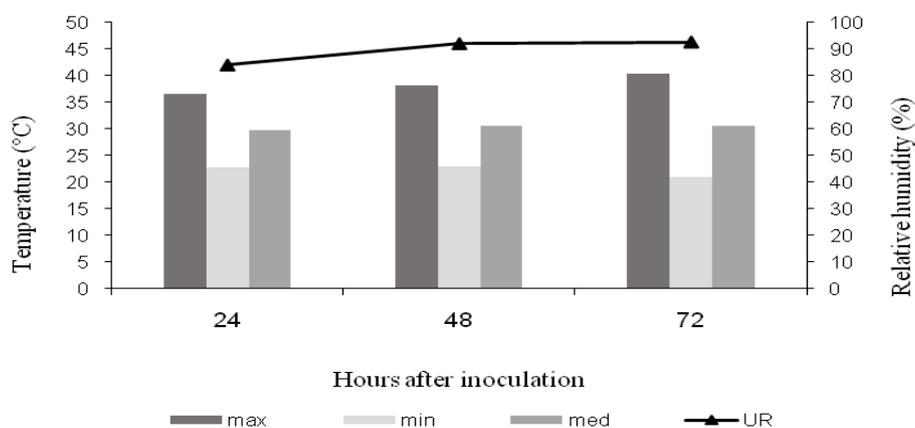
The experimental design was a randomized complete block design with three replications and 22 treatments (six parents, eight F<sub>1</sub> hybrids and eight reciprocal hybrids). In this way, each block was composed of two trays for evaluating resistance, and two trays for the control, ordered by genotype. In one tray, the plants were inoculated

with a GSB isolate grown in a culture medium of potato, dextrose and agar (PDA). In the control tray, only small PDA disks were placed with the plants.

The plot was composed of eight seedlings, arranged in an eight-cell line of the tray. To avoid a microclimate and seedling densification and to facilitate seedling inoculation, cells filled with the same substrate but no plants were alternated between rows in each plot.

The pathogen in its asexual stage (*Phoma cucurbitacearum*) was isolated from melon plants grown in a greenhouse in the Vegetable Crops and Aromatic Medicinal Plants Sector, UNESP-FCAV, Jaboticabal. The isolation of the pathogen was performed directly by removing the pycnidia formed in the lesions of plants with typical symptoms of gummy stem blight. After obtaining the pure fungal culture, sub-culturing was performed in Petri dishes containing PDA culture medium, and 20 days later, 5-mm-diameter medium fragments containing fungus mycelium were removed and used for inoculation.

The plants were inoculated using the toothpick method, when presenting the second true leaf fully expanded. Discs of 5 mm containing the pathogen mycelium were removed from the culture medium, as well as discs containing only culture medium (control). After inoculation, seedlings were kept in a humidity chamber for 72h and then placed in the greenhouse. Figure 1 shows the mean temperature and relative humidity in the chamber during assays.



**Figure 1.** Average temperature and relative humidity at 24, 48, and 72h in a moistened chamber following inoculation of melon with *Didymella bryoniae*. UNESP-FCAV, Jaboticabal, São Paulo State, 2016.

Seven days after inoculation, the severity of infection was scored on a scale from 0 to 4 (0 = no visible symptoms; 1 = up to 1-cm-diameter soaked lesion on the plant stem; 2 = over 1-cm-diameter soaked lesion on the plant stem; 3 = partially necrotic lesion on the stem, with partial wilting of the plant; and, 4 = complete necrosis with total wilting and plant death).

### Statistical analysis

The diallel analysis was performed according to the method proposed by Griffing (1956), adapted to partial diallel by Geraldi and Miranda Filho (1988). The treatment effect (six parents and eight hybrids), considered as fixed, was split into general (GCA) and specific combining (SCA) abilities; the effect of environment was considered random.

The statistical model was:

$$\text{Equation 1: } Y_{ij} = \mu + 1/2(d_1 + d_2) + g_i + g'_j + s_{ij} + \varepsilon_{ij}$$

where:  $Y_{ij}$  is the mean of crossing the  $i$ -th parent of group 1 with the  $j$ -th parent of group 2;  $Y_{i0}$  is the mean of the  $i$ th parent of group 1 ( $i = 0, 1, \dots, p$ );  $Y_{0j}$  is the mean of the  $j$ th parent of group 2 ( $j = 0, 1, \dots, q$ );  $\mu$  is the general average;  $d_1$  and  $d_2$  are the contrasts of the means of groups 1 and 2 and of the general mean;  $g_i$  is the effect of the general combining ability of the  $i$ th parent of group 1;  $g'_j$  is the effect of the general combining ability of the  $j$ -th parent of group 2;  $s_{ij}$  is the effect of the specific combining ability for crosses between the parents of order  $i$ ; and  $\varepsilon_{ij}$  is the experimental error. This linear model was used to obtain hybrid and reciprocal information separately.

The  $p$ -values of the sum of squares of the effects of GCA and SCA were calculated by sum of squares.

There was greater interest in hybrid combinations between resistant and susceptible genotypes; other developments besides the GCA were not explored for combinations of little interest.

The analyses were performed with the aid of the GENES software (Cruz, 2013).

The additive genetic effects involved in character determination were calculated by the relationship between the average squares of the GCA and SCA according to the equation proposed by Beker (1978):

$$\text{Equation 2: } \%CGC = \frac{CGC}{CGC+CEC} \times 100$$

To test whether the effects of GCA and SCA differ statistically from zero, the t-test according to the following formula was applied:

$$\text{Equation 3: } t = \frac{\bar{x}-\mu}{s}$$

where: t = t statistic;  $\bar{X}$  = value of GCA or SCA;  $\mu$  = zero value; and S = standard error of GCA or SCA. The p-value was calculated using a bilateral t distribution and degrees of freedom equal to the number of total diallel crosses.

To select crosses ( $F_1$ s and their reciprocals) highly resistant to *D. bryoniae*, a scatter plot was designed selecting crosses classified with a score less than or equal to one for both  $F_1$ s and reciprocals.

## Results and discussion

There was significant effect for treatments and for the groups of crosses for both  $F_1$ s and their reciprocals (Table 2). The splitting of treatments' degrees of freedom (DF) allowed GCA information to be obtained from each group separately. A significant effect was observed on GCA for G-I and G-II (Table 2). A significant effect was observed on GCA for G-I only for  $F_1$ s. In relation to G-II, there was a significant effect for both  $F_1$ s and their reciprocals (Table 2).

**Table 2.** Analysis of diallel variation for disease severity of *Didymella bryoniae* in melon genotypes. UNESP-FCAV, Jaboticabal, São Paulo State, 2016.

Source of Variation	DF	F <sub>1</sub> s	
		MS	Reciprocal MS
Treatments	13	1.681**	2.009**
Groups	1	9.481**	8.540**
GCA I	1	1.616*	1.064 <sup>ns</sup>
GCA II	3	2.311**	2.831**
SCA	8	0.478 <sup>ns</sup>	1.003**
Residue	26	0.294	0.261
GCA I (%)		36.70	21.70
GCA II (%)		52.50	57.80

\*\* , \* , <sup>ns</sup> Significant at 1% and 5% according to the probability and non-significant according to the F-test, respectively.

No significant effect on GCA was observed for reciprocal G-I parents. This result has little applicability since the parents of this group are susceptible to *D. bryoniae*. However, there was a significant effect on GCA for the G-II parents. The GCA effect indicates the superiority of the parents from the diallel cross and the tendency to add or decrease the average of a given character.

The effect of SCA indicates the complement between the parents in relation to genes with dominant effect. Reciprocal crosses had a significant effect on SCA (Table 2), indicating that the production of hybrids among these parents could influence the probability of obtaining resistant progenies.

Splitting the sum of squares indicated that there is at least one parent superior to the others, in relation to the average performance in hybrid combinations, since the effect of GCA is considered an indicator of the superiority of the parent and its relative divergence among the other parents (Rocha, Stingen, Gemeli, Coimbra, & Guidolin, 2014). This shows that there is a predominance of additive effects in the genetic control of the resistance of melon genotypes to GSB in the G-II parents, expressed by the fact that 52.50 and 57.80% of the variance estimation is composed of GCA or of additive genetic effects involved in determining the characters, for  $F_1$ s and reciprocals, respectively.

The selection of parents for crosses by diallel mating is performed based on estimates of GCA and SCA. It is sought to identify populations containing parents with a high estimate of these capacities, that is, parents that

segregate populations that present the desired mean and genetic variability of significant magnitude. These parameters are fundamental in a population destined to obtain lines (Ramalho et al., 2012).

The GCA estimates for F<sub>1</sub> and reciprocals of the JAB-11 and JAB-20 (G-I) lines show that JAB-20 presents negative and significant estimates, unlike JAB-11 lines (Table 3). This indicates that, in general, crosses with JAB-20 lines may result in individuals with lower mean values and, therefore, a higher possibility of identifying resistant individuals. When reducing the mean of a trait is desired, as in this study, at least one of the parents should have a negative value (Krause, Rodrigues, & Leal, 2012).

Regarding the GCA estimates of the G-II parents for resistance to *D. bryoniae* (Table 3), it must be noted that only PI 420145 and PI 482398 presented negative and significant estimates for F<sub>1</sub> and reciprocals, respectively. These parents may be good candidates for crossbreeding when the purpose of the breeding programme is to increase the resistance of melon genotypes to GSB.

**Table 3.** General combining ability (GCA) estimate for resistance of melon genotypes to *Didymella bryoniae*, for six genitors divided into two groups, used in the partial diallel crosses. UNESP-FCAV, Jaboticabal, São Paulo State, 2016.

Genitors	$\hat{g}_i$			
	F <sub>1</sub>	Pr >  t	Reciprocal	Pr >  t
Group I				
1. JAB-11	0.183	0.0022	0.148	0.0022
2. JAB-20	-0.183	0.0022	-0.148	0.0022
Standard Error (Gi)	0.073		0.073	
Group II				
3. PI 140471	-0.030	0.7120	0.116	0.1581
4. PI 157082	0.514	0.0003	0.507	0.0002
5. PI 420145	-0.278	0.0093	-0.371	0.0015
6. PI 482398	-0.206	0.0337	-0.253	0.0110
Standard Error (Gi)	0.110		0.104	

PI 140471 presented negative estimates of GCA for F<sub>1</sub>s and positive yet low and non-significant estimates for their reciprocals. On the other hand, PI 157082 presented positive estimates of GCA for F<sub>1</sub>s and their reciprocals (Table 3), possibly because the related gene for resistance (*Gsb-2*) is not stable in the inoculum used in the study. Such result indicates that PI 157082 is only slightly resistant.

Considering the effect of SCA for F<sub>1</sub>s and their reciprocals, 12 combinations were observed with negative estimates, of which seven were significant (Table 4). Among the JAB-11 crosses with the G-II parents, the combinations JAB-11 x PI 420145 and JAB-11 x PI 482398 presented negative and significant SCA estimates (Table 4) for F<sub>1</sub>s and their reciprocals, respectively. Thus, these combinations may result in progenies highly resistant to *D. bryoniae*, both in F<sub>1</sub>s and reciprocals. The accession PI 140471 did not show a good combination for genetic complementarity with JAB-11 (JAB-11 x PI 140471).

**Table 4.** Specific combining ability (SCA) of melon genotypes for resistance to *Didymella bryoniae* for six genitors divided into two groups used in the partial diallel crosses. UNESP-FCAV, Jaboticabal, São Paulo State, 2016.

Crosses	$\hat{S}_{ij}$			
	F <sub>1</sub> s	Pr >  t	Reciprocal	Pr >  t
JAB-11 x PI 140471	0.041 <sup>ns</sup>	0.6821	0.628 <sup>**</sup>	0.0002
JAB-11 x PI 157082	0.454 <sup>**</sup>	0.0020	-0.015 <sup>ns</sup>	0.8733
JAB-11 x PI 420145	-0.211 <sup>*</sup>	0.0415	-0.720 <sup>**</sup>	<0.0001
JAB-11 x PI 482398	-0.367 <sup>**</sup>	0.0062	-0.417 <sup>**</sup>	0.0023
JAB-20 x PI 140471	-0.619 <sup>**</sup>	0.0003	-0.772 <sup>**</sup>	<0.0001
JAB-20 x PI 157082	-0.155 <sup>ns</sup>	0.1468	0.133 <sup>ns</sup>	0.1809
JAB-20 x PI 420145	-0.061 <sup>ns</sup>	0.5415	-0.074 <sup>ns</sup>	0.4333
JAB-20 x PI 482398	-0.166 <sup>ns</sup>	0.1235	-0.453 <sup>**</sup>	0.0014
Standard Deviation (Sij)	0.268		0.253	

The accessions PI 140471 and PI 482398 crossed with JAB-20 resulted in the best combinations. The combination JAB-20 x PI 140471 presented high and significant negative SCA estimates (Table 4) for F<sub>1</sub>s and their reciprocals, respectively. The combination JAB-20 x PI 482398 also presented negative estimates; however, they were only significant for the reciprocal. Those results suggest that the use of either male or female parent is suitable for obtaining better results.

Although the SCA estimates of the JAB-20 x PI 420145 cross were negative, they were non-significant and close to zero.

The accession PI 157082 did not present good combinations with JAB-11 and JAB-20; therefore, it is not suggested as a source of resistance to *D. bryoniae*.

Another point to consider in the choice of parents for crosses is the existence of a reciprocal effect. Rocha, Stingen, Gemeli, Coimbra, and Guidolin (2014) suggest that the use of contrasts makes it possible to verify the existence of a reciprocal effect in the genotypes. Therefore, considering that the contrasts between the hybrids and their reciprocals were significant, there is evidence of the reciprocal effect for resistance to GSB in melon evaluated in this work.

There were significant differences in three contrasts between hybrids and reciprocals: (JAB-20 x PI 140471) vs (PI 140471 x JAB-20), (JAB-20 x PI 420145) vs (PI 420145 x JAB-20), and (JAB-20 x PI 482398) vs (PI 482398 x JAB-20), showing a reciprocal effect (REC) on the resistance of melon hybrids to *D. bryoniae* (Table 5).

**Table 5.** Contrasts among means (three replications) of F<sub>1</sub>s and reciprocals (REC) for resistance of hybrids to *Didymella bryoniae* for six genitors divided into two groups used in the partial diallel crosses. UNESP-FCAV, Jaboticabal, São Paulo State, 2016.

Crosses	Contrast		Variance	Pr > t
	F <sub>1</sub> s	REC		
JAB-11 x PI 140471	1.58	vs 2.16	1.430	0.063
JAB-11 x PI 157082	2.54	vs 1.91	0.070	0.672
JAB-11 x PI 420145	1.08	vs 0.33	0.041	0.744
JAB-11 x PI 482398	1.00	vs 0.75	1.581	0.052
JAB-20 x PI 140471	0.55	vs 0.47	5.052	0.001*
JAB-20 x PI 157082	1.56	vs 1.76	0.066	0.681
JAB-20 x PI 420145	0.86	vs 0.68	2.026	0.029*
JAB-20 x PI 482398	0.83	vs 0.42	2.717	0.012*

\*Note: P-values of significant contrasts mean that there is evidence of reciprocal effects.

The reciprocal effects have implications on the estimation of genetic effects. These effects can be further divided into maternal (MAT) and non-maternal (NMAT) effects. Maternal effects are attributable to cytoplasmic genetic factors, and non-maternal effects can be explained by the interaction between nuclear genes and cytoplasmic gene effects (Wu & Matheson, 2001).

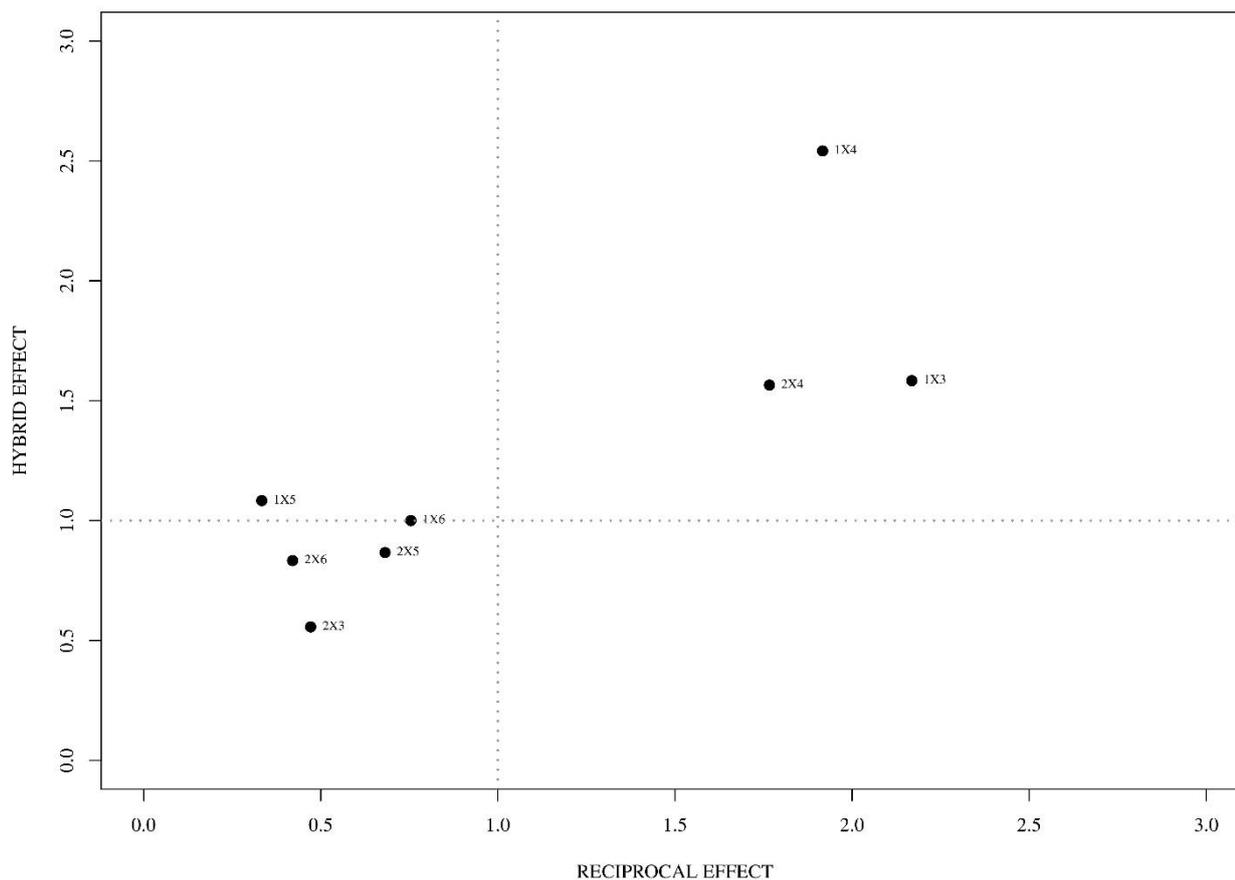
When the reciprocal effect is due to the maternal component, it may persist for generations and can be exploited, but if it is due to the non-maternal component, the effect may be lost over generations. Therefore, as a breeding strategy, it is necessary to consider the nuclear and non-nuclear factors in the selection of plants, as when there is reciprocal effect, it is essential to define which parent will be used as the female or male (Wu & Matheson, 2001).

When reciprocal effects occur, special attention should be given when choosing the best crosses, since the descendant phenotype will be influenced by the maternal genotype. In the present case, there are reciprocal differences for PI 140171, PI 420145 and PI 482398, only when crossed with the parent JAB-20 (Table 5). Thus, these genotypes should be used as female for JAB-20 crosses.

To date, there is no information on the reciprocal effects acting on the resistance of melon genotypes to *D. bryoniae*. However, Amand and Wehner (2001) identified reciprocal differences with cytoplasmic inheritance in crosses between the genotypes M 17 X 'Wis.SMR 18' and 'Slice' x 'Wis.SMR 18' in their study on the resistance of cucumber genotypes to *D. bryoniae*, agreeing with the results obtained in this study.

Considering all the above, the best crosses to obtain lines with resistance to *D. bryoniae* are those where the parents involved, besides presenting negative and significant SCA, also demonstrate a high and stable level of resistance in their hybrids, as well as a reciprocal effect. In this case, crosses that show high resistance should be selected; for GSB specifically, those genotypes with an average symptom score up to 1.0, that is, a lesion size smaller than 1.0 cm should be used.

According to the scatter plot of the mean symptoms of GSB severity for hybrids and their reciprocals (Figure 2), four of the eight hybrid combinations of the partial diallel crosses contributed to the increase in resistance to *D. bryoniae* simultaneously for both the hybrid and the reciprocal effect (quadrant IV), which indicate a higher frequency of expression of resistance alleles; the presence of such combinations also contributes to increasing resistance compared to others, which is reflected by the negative values of SCA in the hybrid combinations of these crosses.



Crosses: 1 x 3 (JAB-11 X PI 140471); 1 x 4 (JAB-11 X PI 157082); 1 x 5 (JAB-11 X PI 420145); 1 x 6 (JAB-11 X PI 482398); 2 x 3 (JAB-20 X PI 140471); 2 x 4 (JAB-20 X PI 157082); 2 x 5 (JAB-20 X PI 420145); 2 x 6 (JAB-20 X PI 482398).

**Figure 2.** Dispersion of means of severity of *Didymella bryoniae* in melon hybrids and respective reciprocals.

The crosses JAB-20 X PI 140471, JAB-20 X PI 420145, and JAB-20 X PI 482398 showed a mean symptom score of < 1.0 for both hybrids and reciprocals (Figure 2). The hybrid combination JAB-20 X PI 140471 showed the lowest mean of symptoms between the hybrids and JAB-20 X PI 482398 among the reciprocal combinations considering the four abovementioned crosses.

Among the crosses with JAB-11 (parent 1), the JAB-11 X PI 482398 cross showed a mean number of symptoms of GSB for the hybrid equal to 1.0 and for the reciprocal < 1.0. Although the JAB-11 X PI 420145 cross presented a mean higher than 1.0, its reciprocal showed a lower mean symptom score among all crosses.

Crosses of the JAB-11 and JAB-20 lines with PI 157082 showed F<sub>1</sub> and reciprocal lines varying from susceptible to moderately resistant. These results reflect the instability of this parent regarding resistance and reinforce the hypothesis that this should be discarded as a reliable source of resistance to *D. bryoniae*.

## Conclusion

The parents PI 420145 and PI 482398 are suggested for crosses in order to obtain melon lines with resistance to *Didymella bryoniae*. The parents PI 482398, PI 420145, and PI 140471 combined with JAB-20 have a reciprocal effect, indicating the existence of a maternal effect. This effect must be considered in the crosses to develop lines with resistance to gummy stem blight. The crosses JAB-11 x PI 420145, JAB-11 x PI 482398, JAB-20 x PI 140471, JAB-20 x PI 420145, and JAB-20 x PI 482398 can generate lines highly resistant to *Didymella bryoniae*.

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