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## Self-incompatibility in passionfruit: evidence of gametophytic-sporophytic control

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**Abstract** Self-incompatibility in passionfruit was studied in families originated from crosses among plants that presented differences in reciprocal crosses. The three families, obtained by crossing  $S_3$  plants, exhibited one incompatible group; no reciprocal differences were observed. The phenotype of the families was the same as the parent plants,  $S_3$ . These results suggest the presence of a gene ( $G$ ), gametophytic in its action, associated to the sporophytic gene  $S$ , modifying the incompatibility reaction in passionfruit. The reciprocal difference exhibited in the crosses among the parents could be explained as a matching between plants homozygous for  $S$ , but homozygous and heterozygous for  $G$ . Actually this would be a partially compatible cross, not detectable when the evaluation is done based on fruit set data. As the family originated from this kind of cross is homozygous for  $S$  and heterozygous for  $G$ , no reciprocal differences are expected, and the phenotype should be the same as the parental plants, as observed in the present work.

**Keywords** Self-incompatibility · Pollination · *Passiflora edulis*

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### Introduction

The self-incompatibility systems are divided into heteromorphic, when there are differences in floral structures, and homomorphic, when differences are absent. The genetic control of the pollen grain phenotype can be gametophytic, in that the phenotype of the pollen grain corresponds to the  $S$  allele it carries, and sporophytic, where the pollen grain phenotype is the same as the plant that produced it (Lewis 1954).

The gametophytic system is well characterized in 15 families, including Solanaceae (Newbigin et al. 1994). The sporophytic system is studied with more emphasis in the Brassicaceae, although it has been described in the Asteraceae, Convolvulaceae (Hinata et al. 1993) and Compositae (Gerstel 1950; Hughes and Babcock 1950).

Bruckner et al. (1995) studied the incompatibility system in passionfruit (*Passiflora edulis*, Passifloraceae), and the results could be fitted to a homomorphic sporophytic system, probably controlled by one gene. The next step was to obtain homozygous families for the different alleles, to study the dominance relationships among them. Surprisingly, Rêgo et al. (1999) observed that new phenotypes were identified in these families, a clear evidence that another gene would be acting.

More than one locus involved in the gametophytic system is related in the Poaceae, the Chenopodiaceae and the Ranunculaceae (Østerbye 1986). In the sporophytic system, only *Eruca sativa* was mentioned, with three loci operating (Verma et al. 1977; mentioned by Lewis 1994).

Lewis et al. (1988) and Zuberi and Lewis (1988) demonstrated the existence of a second gametophytic gene, associated to the sporophytic system, in *Raphanus sativus* and *Brassica campestris*, respectively. The authors believe this is the rule for the Brassicaceae and the sporophytic system in general, and the hypothesis is justified for the absence of inexplicable results in the gametophytic system, and the presence of 2% to 10% of exceptional results in all the species studied in the sporophytic system. According to Lewis (1994), if the

**Table 1** Summary of reciprocal crosses in the progeny BJ<sup>a</sup>

Group (female)	Group (male)			
	XII (14)*	XIII (1)*	XIV (3)*	Indicative plant S <sub>3</sub>
XII (14)*	–	+	–	+
XIII (1)*	+	–	–	–
XIV (3)*	–	+	–	+**
Indicative plant S <sub>3</sub>	+	–	–	

<sup>a</sup>Rêgo et al. (1999)

\* Number of plants in each group; (–): incompatible; (+): compatible; shaded area: difference in reciprocal crossings

\*\* 50% fruit set. Designated as ± by Rêgo et al. (1999)

**Table 2** Name, parents and number of plants evaluated per progeny

Progeny	Parents	Number of plants
CJ	BJ 17 (group XIV) × BJ 20 (group XIII)	9
CK	BJ 21 (group XIV) × BJ 20 (group XIII)	10
CL	BJ 16 (group XIV) × BJ 20 (group XIII)	20

methods were the same for both systems, the non-expected results, found in works with the sporophytic system, are significant, and have to be considered; all these exceptions have been compatible, when an incompatible result was expected. Unexpected positive results were also found in the passionfruit (*P. edulis*). Bruckner et al. (1995) found 4.2% positive results (5 to 11.1% of fruit set) in the progeny 300 and 1.4% (20% of fruit set) in the progeny 414,414. Rêgo et al. (1999) obtained 10 to 50% fruit set in 1.4 to 2.9% of the crossings made in some of the families studied.

Differences in reciprocal crossings in the *Passiflora* were mentioned by Akamine and Girolami (1959), Knight and Winters (1962), Chang (1974) and Rêgo et al. (1999). Of those observed by Rêgo et al. (1999), the more interesting ones were in the progeny BJ. The plants of self-incompatible group XIV were compatible when were crossed with plants of self-incompatible group XIII, and the indicative plant (phenotype S<sub>3</sub>), whereas the reciprocal crossing was incompatible (Table 1).

The objective of this work was to study the inheritance of self-incompatibility in the passionfruit, in progenies of crossings among plants of the BJ family, obtained by Rêgo et al. (1999), that presented differences in reciprocal crossings.

## Materials and methods

The experiment was conducted in the Department of Phyto-technics, in the Federal University of Viçosa, Viçosa-MG, from November 1996 to February 1999. The progenies used in this work (Table 2) were obtained from crossings in the progeny BJ (Rêgo et al. 1999), involving plants of the groups XIII and XIV, that presented differences in reciprocal crossing.

Pollination was done as described by Bruckner and Otoni (1999). Five pollinations, at least, were made for each crossing. The pollination was considered compatible when there was fruit set (+), or incompatible (–) in the absence of the same.

**Table 3** Results of reciprocal crosses in the progeny CJ (BJ17 × BJ20). (–): incompatible (no fruit set); \*: fruit set, percentage

Female	Male									
	9	11	4	5	3	7	13	8	12	
9	–	–						–		
11	–	–	–			–				
4	–	31.25*	–	–		–				
5	–	–	–	–	–					
3				–	–	–			–	
7		–		–	–	–	–			
13						–	–	25*		
8	–	–					37.5*	–		
12		–							–	

**Table 4** Results of reciprocal crosses in the progeny CK (BJ21 × BJ20). (–): incompatible (no fruit set); \*: fruit set, percentage

Female	Male									
	4	1	2	7	10	12	13	9	16	18
4	–	–	–				–			
1	–	–	–							
2		–	–	28.57*	–					
7	–	–	–	–	–					
10	–			–	–	–				
12				–	–	–	–			
13							–	–		
9	–						–	–		
16								–	–	
18		–							–	

Reciprocal crossings have been made, crossing the first plant of the progeny with the second, the second with the third, successively, until the last plant, forming a chain (Wallace 1979). When the results of the crossings were incompatible, the plants were considered to be of the same phenotype; in the case of compatibility, the plants were considered to be of different groups, with different phenotypes.

## Results

The majority of the crossings in the progeny CJ were incompatible (Table 3). In some cases, there was a certain rate of fruit set: 31.25% within CJ4 × CJ11, while the reciprocal was incompatible; 25% within CJ13 × CJ8 and 37.5% in the reciprocal crossing. Crossings involving CJ11

**Table 5** Results of reciprocal crosses in the progeny CL (BJ16 × BJ20). (-): incompatible (no fruit set); \*: fruit set, percentage

Female	Male																			
	10	9	8	7	11	13	6	4	5	3	2	12	14	15	16	18	19	20	21	22
10	-	-																		
9	-	-																		
8		-	-																	
7			-	-																
11				-	-						-									
13					-	-														
6						-														
4							-													
5								-												
3									-											
2										-										
12											-									
14												-								
15													-							
16														-						
18															-					
19																-				
20																	-			
21																		-		
22																			-	

and other plants of the progeny CJ (CJ5, CJ7, CJ8, CJ9 and CJ12) were incompatible, which allows us to consider that these plants have the same phenotype. The same occurred with plant CJ8; its crossings with other plants of the progeny (CJ9 and CJ11) were clearly incompatible. The progeny CJ can be considered as a unique group.

The progeny CK (Table 4) gave fruit set in just one crossing, 28.75% within CK2 × CK7, being its reciprocal incompatible. The crossings between CK7 and other plants of the progeny (CK4, CK10 and CK12) were also incompatible. The plants of the progeny CK can be considered to be of the same phenotype.

These observed values of fruit set are relatively low, up to 31.25% in the progeny CJ and 28.57 in CK. Stevens and Kay (1988) reported differences if the evaluation of self-incompatibility is done by counting the number of seeds (similarly to fruit set), or cytologically (observing the pollen grains in the stigma, under a microscope). Compatible crossings and failure in the incompatible reaction results in fruit set, which can account for erroneous conclusions.

Hatakeyama et al. (1998) studied the dominance relationships among the *S* alleles in *Brassica oleracea*, observing pollen grains in the stigma. They verified that some crossings presented inconsistent results, that could sometimes be considered compatible, and others as incompatible. The authors classified these crossings as “indeterminable”, and interpreted them as an eventual failure in expression of the incompatibility.

In the progeny CL, all the crossings were incompatible, without exception, and the 20 plants could be clustered into one group (Table 5).

Plants of the three progenies were crossed to each other and with the indicative plant  $S_3$  to identify the phenotype and, in agreement with the results, CJ, CK and CL possess the same phenotype,  $S_3$  (Table 6).

**Table 6** Crosses among plants of the progenies CJ, CK and CL and the indicative plant. (-): incompatible; \*: indicative plant  $S_3$  probably homozygous ( $S_3 S_3$ ) (Rêgo et al. 1999)

Female parent	Male parent						
	CJ5	CJ11	CJ13	CK2	CK4	CK9	BC3*
CJ5				-			
CJ11				-			
CJ13							
CK2		-					
CK4	-						
CK9		-	-				
CL10						-	
CL12						-	
CL13						-	
BC3*		-			-		

## Discussion

Differences in reciprocal crossings are common in the sporophytic system (Lewis 1954) and can be explained by: (1) differences in dominance relationships among the alleles in the pollen and in the pistil, as related for *Sinapis arvensis* by Steven and Kay (1988); and (2) a gametophytic gene associated with the sporophytic system, verified in *Raphanus sativus* and *Brassica campestris* by Lewis et al. (1988) and Zuberi and Lewis (1988), respectively.

According to Hatakeyama et al. (1998), in Brassica the codominance occurs more frequently in the female genitor. In that case, the heterozygous plant can fertilize a homozygous plant for the recessive allele, but the reciprocal crossing is incompatible, because of the codominance action of the recessive allele.

On the other hand, when the differences in reciprocal crossings are caused by a gene of gametophytic action

**Table 7** Presumed genotypes of groups XIII and XIV of the progeny BJ, and the indicative plant (Rêgo et al. 1999). (-): incompatible; (+): compatible; shaded area: difference in reciprocal crossings

Group (female)	Group (male)			
		XIII $S_3S_3 G_1G_2$	XIV $S_3S_3 G_1G_1$	Indicative plant $S_3$ $S_3S_3 G_1G_2$
	Genotype			
XIII	$S_3S_3 G_1G_2$	-	-	-
XIV	$S_3S_3 G_1G_1$	+	-	+
Indicative plant $S_3$	$S_3S_3 G_1G_2$	-	-	-

(G), the crossing only occurs when the female plant is homozygous for G, and the male is heterozygous. In that case, just half of the pollen (the one that contains the different allele) can fertilize the female homozygous plant, giving rise to heterozygous progeny for G. In this progeny, therefore, differences in reciprocal crossings are not expected (Lewis et al. 1988). In backcrossings to the female parent, on the other hand, these differences are expected, being viable in crossings when the female parent receives pollen.

The results indicate that all the plants of the progenies CJ, CK and CL are  $S_3$ . The group XIII of the progeny BJ, represented only by the plant BJ20, which was the male genitor of CJ, CK and CL also presented the  $S_3$  phenotype. The phenotype of plants BJ16, BJ17 and BJ21, that formed group XIV, and are the female parents of the progenies used in this work, could only be considered  $S_3$  when those plants were pollen donors (Rêgo et al. 1999). Crossing among plants of groups XIII and XIV was only compatible when the plant of group XIII was the pollen donor, the reciprocal crossing being incompatible (Rêgo et al. 1999). It can be presumed that the group XIV, found by Rêgo et al. (1999), was also  $S_3$  and that, probably, a second gene G, gametophytic, exists associated to the sporophytic system in the passionfruit. It was not possible, though, to accomplish backcrossings with those progenies, because parents no longer existed.

Considering that the groups XIII and XIV of the progeny BJ (Table 1), evaluated by Rêgo et al. (1999), have the same phenotype ( $S_3$ ), summing four plants, and that the group XII, that had 14 plants, was classified as phenotype  $S_6$ , it can be inferred that  $S_6$  is dominant over  $S_3$  and that the plants of groups XIII and XIV are homozygous for the allele  $S_3$ .

Lewis (1994) mentioned the inhibition of the pollen tube in the intermediate part of the stigma in species with homomorphic sporophytic incompatibility in the Compositae. These reactions are denominated by the author as P\* reactions, and were found in crossings that also presented reactions of the type +\-, characterized as partially compatible in the gametophytic system. This kind of reaction occurs when the plants involved in certain crossing possess one allele in common. Half of the pollen grains (common) are inhibited (-), while the other half accomplishes fertilization (+).

In self-pollinations and incompatible crossings in passionfruit, Rêgo et al. (2000) verified that the inhibition occurred in the surface of the stigma, a characteristic of the sporophytic system, as was already described by Ho and Shii (1986). Examining the site of pollen inhibition in the

plants of progeny BJ, that exhibited differences in reciprocal crossing, Rêgo et al. (2000) verified that, in the incompatible crossing, the growth of the pollen tube was interrupted in the middle part of the stigma, as usually happens in the gametophytic system (reactions that can be considered of type P\*). Unfortunately, these authors did not accomplish restricted pollination to identify the presence of partially compatible reactions. According to Lewis (1994), reactions of the type P\* segregate in association with reactions of type +\-, which is the strongest evidence that a second locus, with gametophytic action, is actuating.

Knight and Winters (1962), studying the inhibition of the growth of the pollen tube in crosses among some passionfruit clones, verified the occurrence of a partially compatible crossing: 1-32  $\times$  3-32 (Table 4 of the referred paper). The authors commented on the existence of some developed pollen tubes, while others clearly had been inhibited, and named the reaction as compatible/incompatible (mixed).

The plant genotypes of the progeny BJ can be interpreted as  $S_3S_3 G_1G_2$  for the plant BJ20 (group XIII), and  $S_3S_3 G_1G_1$  for the plants BJ16, BJ17 and BJ21 (group XIV), as represented in Table 7. When BJ20 was pollinated with BJ16, 17 or 21, an incompatible reaction occurred because, considering BJ20 heterozygous for G, the two alleles  $G_1$  and  $G_2$  were inhibited. The reciprocal crossing was compatible because the plants  $G_1G_1$  had the allele  $G_1$  in common with BJ20. Actually it is assumed that the reaction was partially compatible (+\-), but evaluated as compatible considering fruit setting. The plant BJ17 (group XIV), evaluated by Rêgo et al. (1999), presented differences in the incompatibility reaction in the reciprocal crossings with the indicated plant, according to the data summarized in Table 3. This suggests that the indicative plant was also  $S_3S_3 G_1G_2$ .

The progenies obtained from the crossings between BJ20 and BJ16, BJ17 and BJ21, studied here, would be  $S_3S_3 G_1G_2$  and there would not be +\- reactions among them. However, if the compatibility differences in reciprocal crossings were due to the codominance among S alleles, these differences would be also present in these progenies.

Lewis et al. (1988) and Zuberi and Lewis (1988), who introduced the hypothesis of a second gene of gametophytic action, associated to the locus S in the sporophytic system, commented that the gene G would be expressed in the presence of dominant S alleles. The same authors verified that the gene G was expressed in more than one allelic combination, and considered the possibility that it was also expressed in the presence of more recessive S alleles.

According to Lewis (1994), the more recent results in the investigations on the incompatibility system of *Eruca sativa* (the unique species in the sporophytic system in which self-incompatibility was governed by three *S* loci) indicate the presence of a second gene *G*, substituting the theory previously proposed by Verma et al. (1977) mentioned by Lewis (1994).

In recent years, as a result of molecular studies, the components of the *S* locus in the stigma (SLG and SRK) as well as in the pollen (SCR) were identified (Franklin-Tong and Franklin 2000). The mode of action of those molecules follows the model proposed by Bateman, in the fifties, where the products of the gene *S* would interact with the surface of the stigma and the outer layer of the pollen grain coat, unchaining a reaction that would result in the rejection of the incompatible pollen grain (Dixit and Nasrallah 2001). Surprisingly, the expression of the gene that codifies SCR is gametophytic (Schopfer et al. 1999). Doughty et al. (1998), in an attempt to identify the component of the *S* locus in the pollen grain, verified that the expression of a protein related with the outer layer of the pollen grain coat (PCP-A1) was also gametophytic. For those authors, that fact opened the possibility that gametophytic expression could be the rule for the genes *S* of the pollen grain, even when they exhibit a sporophytic phenotype. According to them, this would be possible with the synthesis and secretion of those proteins during the development of the pollen grain, and with their incorporation mixed in the pollen grain coat, conferring the apparent sporophytic phenotype. This model can explain the gene *G* action associated with the gene *S*.

The genes *S* and *G* do not completely explain the inheritance of self-incompatibility in the passion fruit plant. The segregation of phenotypes in the progenies BA, BD and BG, related by Rêgo et al. (1999), cannot be explained by the action of the genes *S* and *G*, only. Other genes must be acting in the expression of the character. The new phenotype, *S*<sub>5</sub> found by Rêgo et al. (1999) in the progeny BD group VII, should be discarded, or would be considered as *S*<sub>3</sub>*S*<sub>3</sub> *G*<sub>1</sub>*G*<sub>1</sub>, equal to group XIV of the progeny BJ. The plant of this group was considered compatible with the indicative plant *S*<sub>3</sub>. However, it received pollen of the plant (*S*<sub>3</sub>*S*<sub>3</sub> *G*<sub>1</sub>*G*<sub>2</sub>), lacking the reciprocal crossing. Moreover, the phenotype *S*<sub>5</sub> was not identified in any of the progenies in the investigations conducted by Bruckner et al. (1995) and Rêgo et al. (1999).

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## References

- Akamine EK, Girolami G (1959) Pollination and fruit set in the yellow passion fruit. Hawaii. Agric Exp Sta Techn Bull 39
- Bruckner CH, Otoni WC (1999) Hibridação em maracujá. In: Borém A (ed) Hibridação Artificial de Plantas, UFV, Viçosa, pp 379–399
- Bruckner CH, Casali VWD, Moraes CF, Regazzi AJ, Silva EAM (1995) Self-incompatibility in passion fruit (*Passiflora edulis* Sims). Acta Hort 370:45–52
- Chang CC (1974) Studies on the cause of unfruitfulness of yellow passion fruit flowering in Taiwan (in Chinese). Taiwan Agri Quart (Tai-Wan nung yeh) 10:78–79
- Dixit R, Nasrallah JB (2001) Recognizing self in the self-incompatibility response. Plant Physiol 125:105–108
- Doughty J, Dixon S, Hiscock SJ, Willis AC, Parkin IA, Dickinson HG (1998) PCP-A1, a defensin-like *Brassica* pollen coat protein that binds the *S* locus glycoprotein, is the product of gametophytic gene expression. Plant Cell 10:1333–1347
- Franklin-Tong VE, Franklin CH (2000) Self-incompatibility in *Brassica*: the elusive pollen *S* gene is identified! Plant Cell 12:305–308
- Gerstel DU (1950) Self-incompatibility studies in guayule II. Inheritance. Genetics 35:482
- Hatakeyama K, Watanabe M, Takasaki T, Ojima K, Hinata K (1998) Dominance relationships between *S*-alleles in self-incompatible *Brassica campestris* L. Heredity 80:241–247
- Hinata K, Watanabe M, Toriyama K, Isogai A (1993) A review of recent studies on homomorphic self-incompatibility. Int Rev Cytol 143:257–296
- Ho WF, Shii CT (1986) Incompatibility system in passion fruit (*Passiflora edulis* Sims). Acta Hort 194:31–38
- Hughes MB, Babcock EB (1950) Self-incompatibility studies in guayule. II. Inheritance. Genetics 35:482–506
- Knight Junior RJ, Winters HF (1962) Pollination and fruit set of yellow passion fruit in southern Florida. Florida State Hort Soc 75:412–418
- Lewis D (1954) Comparative incompatibility in angiosperms and fungi. Adv Genet 6:235–285
- Lewis D (1994) Gametophytic-sporophytic incompatibility. In: Williams EG, Clarke AE, Knox RB (eds) Advances in cellular and molecular biology of plants. Genetic control of self incompatibility and reproductive development in flowering plants. Dordrecht: Kluwer Academic Publishers, v 2, pp 88–101
- Lewis D, Verma SC, Zuberi MI (1988) Gametophytic-sporophytic incompatibility in the Cruciferae – *Raphanus sativus*. Heredity 61:355–366
- Newbigin E, Anderson MA, Clarke AE (1994) Gametophytic self-incompatibility in *Nicotiana glauca*. In: Williams EG, Clarke AE, Knox RB (eds) Advances in cellular and molecular biology of plants. Genetic control of self incompatibility and reproductive development in flowering plants. Kluwer Academic Publishers, Dordrecht, v 2, pp 5–18
- Østerbye U (1986) Self-incompatibility in *Ranunculus acris* L. III. *S*-loci numbers and allelic identities. Hereditas 104:61–73
- Rêgo MM, Bruckner CH, Silva EAM, Finger FL, Siqueira DL, Fernandes AA (1999) Self-incompatibility in passionfruit: evidence of two locus genetic control. Theor Appl Genet 98:564–568
- Rêgo MM, Rêgo ER, Bruckner CH, Silva EAM, Finger FL, Pereira KJC (2000) Pollen tube behavior in yellow passion fruit following compatible and incompatible crosses Theor Appl Genet 101:685–689
- Schopfer CR, Nasrallah ME, Nasrallah JB (1999) The male determinant of self-incompatibility in *Brassica*. Science 286:1697–1700
- Stevens JP, Kay QON (1988) The number of loci controlling the sporophytic self-incompatibility system in *Sinapis arvensis* L. Heredity 61:411–418
- Verma SC, Malile, R, Dhir I (1977) Genetics of the incompatibility system in the crucifer *Eruca sativa* L. Proc R Soc Lond Ses B, pp 131–159
- Wallace DH (1979) Procedures for identifying *S*-allele genotypes of *Brassica* Theor Appl Genet 54:249–265
- Zuberi MI, Lewis D (1988) Gametophytic-sporophytic incompatibility in the Cruciferae – *Brassica campestris*. Heredity 61:367–377