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M. M. do Rêgo · C. H. Bruckner · E. A. M. da Silva
F. L. Finger · D. L. de Siqueira · A. A. Fernandes

Self-incompatibility in passion fruit: evidence of two locus genetic control

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Abstract The self-incompatibility in yellow passion fruit was previously described as homomorphic sporophytic with monofactorial inheritance. Five progenies were obtained by bud-selfing. The plants of these progenies were selfed, reciprocally crossed within each progeny and crossed with known incompatible phenotypes to identify their phenotypic group. Fruit set was evaluated at the 7th day after pollination. Two progenies consisted of two self-incompatible groups, the other three formed three suck groups. The groups were identified as S_1, S_2, S_3, S_4, S_5 and S_6. The results provide evidence that the self-incompatibility of passion fruit is controlled by two loci, the S-gene and another, whose expression needs to be investigated.

Key words *Passiflora* · Self-incompatibility · Genetics

Introduction

The main passion fruit species with edible fruits are *Passiflora edulis* Sims., *P. edulis* f. *flavicarpa* Deg., *P. ligularis* Juss., *P. molissima* (HBK) Bailey and *P. quadrangularis* L. (Martin and Nakasone 1970). The most important species is *P. edulis* f. *flavicarpa*, also known as yellow passion fruit. Pollination is one of the most important factors affecting fruit set in the yellow passion fruit, and is carried out by pollinating agents such as carpenter bees, *Xylocopa* ssp. (Akamine and Girolami 1959). The flowers are perfect, hermaphrodite and self-incompatible.

Self-incompatibility is very frequent in nature. In cultivated species, it becomes less frequent, due to the contrary selection pressure, caused by domestication (Mather 1953; Rowlands 1964; de Nettancourt 1977). Although the incompatibility among plants within self-incompatible populations of *Passiflora* was reported by Munro as long ago as 1868 (cited in de Nettancourt 1977), references relating to the incompatibility system and its genetic control are more recent. Akamine and Girolami (1959), Knight and Winters (1962), and Chang (1974) obtained differences of fruit set in reciprocal crosses. Ho and Shii (1986) verified that the incompatible reaction takes place at the papillary cells of the stigma.

Bruckner et al. (1995) studied self-incompatibility in progenies over two generations. In the first generation, two progenies were obtained after crossing among three randomly selected plants. The second generation was obtained from crosses between plants of the progenies. One progeny was produced by self-pollination. The authors concluded that the self-incompatibility of passion fruit is homomorphic and sporophytic. Three alleles were identified: S_1, S_2 and S_3. S_2 was found to be dominant over S_1 and S_3. The results were in agreement with a single S-gene hypothesis. In the same work it was found that self-pollination is possible when flowers at the bud stage are pollinated with pollen from open flowers of the same plant. In one of the studied progenies (414.414) a new and unexpected self-incompatible phenotype was found (C. H. Bruckner, personal communication). The new phenotype (plant 414.414-10) was provisionally named S_4. To analyze

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M. M. do Rego
Campus de Paricarana, Universidade Federal de Roraima, 69300-270 Boa Vista-RR, Brazil

C. H. Bruckner (✉) · F. L. Finger · D. L. de Siqueira · A. A. Fernandes
Departamento de Fitotecnia, Universidade Federal de Viçosa, 36571-000-Viçosa-MG, Brazil

E. A. M. da Silva
Departamento de Biologia Vegetal, Universidade Federal de Viçosa, 36571-000 Viçosa-MG, Brazil

this finding, the plant 414.414-10 was selfed, backcrossed and sib-crossed. The offspring of these crosses and another selfed progeny were studied with the aim of elucidating the inheritance of self-incompatibility in passion fruit.

Materials and methods

The studies were conducted at the Department of Phytotechnics, Federal University of Viçosa, during 1995 and 1996. The crosses and self-pollinations were done within the progenies BA, BB, BD, BG and BJ. These progenies were obtained after self pollination at the bud-stage (Bruckner et al. 1995). The progeny BA originated by selfing the plant 414.414-10, which derived from plant 414 by selfing. The progeny BB was obtained by self-fertilization of the plant Y_4, phenotype S_4. The progeny Y resulted from the cross between plant 414 (phenotype S_2) and 414.414-10 (S_4). The progeny Y showed the phenotypes S_1 (plant Y_1), S_2 (Y_5) and S_4 (Y_3 and Y_4). The progeny BD resulted from self-fertilization of the plant A_4 (phenotype S_4). The progeny A derived from the cross between 414.414-3 (S_2) and 414.414-10 (S_4). The phenotypes of the A progeny are S_2 (A_1 and A_3) and S_4 (A_4). The progeny BG originated from selfing the plant B_3 (unknown phenotype). The B progeny resulted from the backcross of plant 414.414-10 (S_4) to 414 (S_2). The B progeny had the phenotypes S_2 (B_1, B_2 and B_5) and B_3, of unknown phenotype (the plant died before it was identified) but which was not S_2 nor S_4. Progeny BJ was derived from selfing the plant 808, which is not related to the others.

All plants of the progenies were selfed with the aim of confirming the self-incompatibility. Five flowers per plant were protected in the morning with a paper bag, self pollinated at 1 PM or later, and again protected. After 7 days, the fruit set was evaluated. The compatibility among the plants within progenies was evaluated in reciprocal crosses. Each plant of the progenies was reciprocally crossed to the next one in the row, forming a chain of reciprocal crosses (Wallace 1979 b). The plants in which crosses were incompatible were grouped as self-incompatible groups. Additional crosses were made when the crosses between adjacent plants were compatible. In each case, five or more flowers were crossed. Fruit set was evaluated after 7 days. The crosses were classified as compatible, when fruit set occurred, or incompatible, in the absence of it. With the purpose to identify their phenotypes, some plants of the self-incompatible groups within each progeny were crossed with known phenotypes (Table 1). Incompatible crosses indicated that the plants belonged to the same phenotype.

Table 1 Incompatibility phenotypes in passion fruit

Phenotype	Plants	Dominance relationships
S_1	Y_1	Recessive to S_2[a]
S_2	A_1, A_3	Dominant over S_1 and S_3[a]
S_3	BC_3, BC_5, BC_7	Recessive to S_2[a]
S_4	Y_3, Y_4	

[a] Bruckner et al. (1995)

Results

Self-pollinations, made in progenies BA, BB, BD, BG and BJ, did not set fruit, indicating that all plants were self-incompatible. These progenies had yellow fruits. Ruggiero et al. (1978) and Bruckner et al. (1995) described similar results in Brazil. In Hawaii, Akamine and Girolami (1959) found an average of 0.96% fruit set after selfing. Knight and Winters (1962), in Florida, obtained rates between 3 and 20.4%. Chang (1974), in China, verified 4% fruit-set rates. These results indicate the possibily presence of modifying genes. The extent of self-incompatibility could also be low due to highly recessive alleles, as verified in Brussels sprouts (Johnson and Blyton-Conway 1976).

Two self-incompatible groups were found in the BA progeny, groups I and II (Table 2). One anomalous result was also found in group I. The cross 2 × 10 resulted in less than 10% fruit set. The production of 11 plants in group I and three in group II could be explained by a monofactorial hypothesis. Groups I and II were identified as phenotypes S_4 and S_3, respectively (see Table 7). The fact that the S_4 phenotype, the same as that of plant 414.414-10, was more frequent in the progeny, could indicate a dominance of allele S_4 over S_3. However, S_3 and S_4 would not be expected in this progeny in terms of its derivation.

Table 2 Results of reciprocal crosses among 14 plants of progeny BA, classified in two self-incompatible groups. Compatible (+) and incompatible (−) crosses and about 10% fruit set (±)

Male	Group I											Group II		
Female	1	2	3	5	6	10	11	12	17	19	20	8	9	13
1	−	−		−		−								+
2	−	−	−			±								
3	−		−	−										
5			−	−										
6				−	−							+		
10	−				−	−	−	−					+	
11						−	−							
12	−							−						+
17									−	−				
19									−	−	−			
20	−									−	−			
8					+							−	−	−
9						+						−	−	−
13								+				−	−	−

Table 3 Results of reciprocal crosses among ten plants of progeny BB, classified in two self-incompatible groups (III and IV). Compatible (+) and incompatible (−) cross

Male	Group III						Group IV			
Female	1	8	9	13	27	31	34	35	39	40
1	−	−					+		+	+
8	−	−	−							
9		−	−	−			+	+		
13		−	−	−						
27			−	−	−	−				
31	−		−		−	−	+	+	+	
34	+		+			+	−	−		
35			+			+	−	−	−	
39							−	−	−	−
40			+			+			−	−

Table 4 Results of reciprocal crosses among 11 plants of progeny BD, classified in tree self-incompatible groups (VI, VII and VIII). Compatible (+) and incompatible (−) cross and 20% fruit set (±)

Male	Group VI					Group VII			Group VIII		
Female	1	4	7	8	11	5	10	17	12	15	16
1	−	−									
4		−	−			+		+			
7		−	−	−	−	+					
8			−	−		+	+			+	
11			±		−		+		+	+	
5		+	+			−			+		
10			+	+	+		−			+	
17	+	+						−		+	+
12			+		+				−	−	
15				+	+		+	+	−	−	−
16								+		−	−

Two self-incompatible groups were found in progeny BB, groups III and IV, with six and four plants, respectively (Table 3). Group III was identified as S_4 and group IV as S_1 (see Table 7). These results are in agreement with the monofactorial hypothesis.

After reciprocal crosses, the plants of progeny BD were classified into three groups, VI, VII and VIII (Table 4). One anomalous result was observed in the cross 11 × 7. Groups VI and VIII were identified as phenotypes S_4 and S_1, respectively (see Table 7). Group VII is a new phenotype, named S_5. These results would not be expected on a monofactorial hypothesis.

The results of the reciprocal crosses in BG progeny lead to the occurrence of three self-incompatible groups, IX, X and XI (Table 5). In group IX one anomalous result was observed, with 37.5% fruit set after crossing plants 24 and 26. Reciprocal differences were observed between groups IX and X. The crosses 24 × 30, 24 × 34 and 31 × 32 were incompatible, while their reciprocals were compatible. The cross 35 × 24 was incompatible, but their reciprocal was not made. Groups IX, X and XI were identified as S_1, S_4 and S_3, respectively (see Table 7). These results would not be expected on the monofactorial hypothesis, as earlier proposed (Bruckner et al. 1995). It is important to note that the phenotype S_3 reappeared after self-fertilization of the plant B_3 (unknown phenotype), which originated from the backcross of a S_4 plant (414.414-10) to its parent 414 (S_2). These results provide strong evidence that another gene controls self-incompatibility in the passion fruit.

In BJ progeny, the 18 plants were classified as three self-incompatible groups, XII, XIII and XIV (Table 6). Two anomalous results were observed within group XIII, by crossing the plants 2 × 1 and 7 × 8. The crosses among the plants of groups XIII and XIV were compatible when the plants of group XIII were used as the

Table 5 Results of reciprocal crosses among 15 plants of progeny BG, classified in the tree self-incompatible groups (IX, X and XI). Compatible (+) and incompatible (−) cross and 35.7% fruit set (±)

Male	Group IX							Group X				Group XI			
Female	2	6	10	12	24	26	32	30	31	34	35	7	9	33	36
2	−				−										
6		−			−					−					
10			−	−	−										
12			−	−	−										
24	−	−	−	−	−	±	−	−	+	−		+	+	+	+
26					−	−									
32					−		−			+					+
30					+			−	−						
31					+		−	−	−	−				+	
34					+				−	−	−			+	
35					−					−	−				
7		+			+	+					+	−	−	−	
9			+		+							−	−		−
33					+		+			+				−	
36					+				+		+			−	−

Table 6 Results of reciprocal crosses among 18 plants of progeny BJ, classified in three self-incompatible groups (XII, XIII and XIV). Compatible (+) and incompatible (−) crosses and 50% fruit set (±)

Male / Female	Group XII														Group XIII	Group XIV		
	1	2	3	5	6	7	8	10	11	12	13	14	15	23	20	16	17	21
1	−	−	−											−				
2	±	−	−											−	+			
3	−	−	−	−		−												
5			−	−	−													
6				−	−	−												
7			−		−	−	±											
8						−	−	−										
10							−	−	−									
11								−	−	−								
12									−	−	−							
13										−	−	−						
14											−	−	−				+	
15												−	−		+	+		
23	−	−											−					+
20		+											+			−	−	−
16													+		+	−	−	
17												+			+	−	−	−
21														+	+		−	−

male parent, and incompatible in the reciprocal crosses. Group XIII was identified as S_3 (Table 7), and group XII as a new phenotype, named S_6. Group XIV needs additional investigation, since it gives the same pattern as group XIII (S_3) when used as a male parent. The plants with the new phenotypes S_5 (group VII, progeny BD) and S_6 were compatible in crosses, which indicates that they are different.

Discussion

Akamine and Girolami (1959), Knight and Winters (1962), and Chang (1974) all reported differences in reciprocal crosses in passion fruit. According to Lewis (1954), such differences are characteristic of the sporophytic system. The results of Akamine and Girolami (1959), Knight and Winters (1962), and Chang (1974) also showed considerable variation in fruit set, which can be attributed to variable ambient conditions, such as rainfall, temperature and photoperiod at the time of crossing. Bruckner et al. (1995) observed little difference in reciprocal crosses, and attributed the observed variability to randomness. A possible hypothesis to be investigated is the presence of an independent gene, whose expression depends on an allele of the S series. Lewis et al. (1988) and Zuberi and Lewis (1988), studying differences in reciprocal crosses in *Brassica*, reported unexpected compatible crosses probably due to a gametophytic gene associated with the sporophytic monofactorial system, whose expression occurs only when associated with particular genotypes of the sporophytic system.

In the present work, small fruit-set rates were observed in some intra-group crosses in the progenies BD (Table 4), BG (Table 5) and BJ (Table 6). In all cases the reciprocal crosses did not show fruit set. In inter-group crosses, differences in reciprocal crosses were detected in the progenies BG (Table 5) and BJ (Table 6). BG_{24} (group IX) was cross compatible with BG_{30} and BG_{34} (group X) only if used as the male parent. The crosses $BG_{35} \times BG_{24}$ and $BG_{31} \times BG_{32}$ were also incompatible, but the reciprocal crosses could not be done. In the BJ progeny, all crosses of BJ_{20} (group XIII) with the plants of group XIV (BJ_{16}, BJ_{17} and BJ_{21}) were compatible when BJ_{20} was used as the male parent and incompatible in the reciprocal crosses. Studies of pollen-tube development in passion fruit demonstrated that in incompatible crosses the pollen tubes ceased to grow normally at the papillary cells of the stigma (Ho and Shii 1986; Rêgo et al. 1996), this beeing a characteristic of the sporophytic system (Heslop-Harrison and Shivanna 1977; de Nettancourt 1977). In the BG and BJ progenies, the pollen-tube development of crosses with reciprocal differences were studied (Rêgo et al., unpublished). In these incompatible crosses, the growth of the pollen tube ceased in the style tissue, as observed in gametophytic systems (Heslop-Harrison 1975). These results suggest the presence of a gametophytic gene that acts in association with the S-gene in *Passiflora*. This hypothesis needs to be investigated.

Table 7 Results of reciprocal crosses of plants of the self-incompatible groups with plants of known S-phenotypes. Compatible (+) and incompatible (−) crosses. Male (↓) and female (↑) parent

Group	Plant	Known S-phenotypes							
		S_1		S_2		S_3		S_4	
		↓	↑	↓	↑	↓	↑	↓	↑
I	BA$_1$	+	+	+		+		−	
	BA$_2$	+						−	
	BA$_5$	+						−	
	BA$_{20}$	+						−	−
II	BA$_8$	+	+	+	+	−	−	+	
	BA$_{13}$	+	+	+	+				
III	BB$_1$	+		+		+	+	−	−
	BB$_9$			+	+	+		−	−
	BB$_{13}$			+	+	+			
IV	BB$_{35}$			+	+				
	BB$_{40}$	−	−			+	+		
VI	BD$_7$					+			
	BD$_8$	+						−	−
VII	BD$_5$							+	
	BD$_{10}$	+	+	+				+	+
	BD$_{17}$			+	+	+			
VIII	BD$_{15}$	−	−	+		+		+	
IX	BG$_{24}$	−	−	+	+	+		+	
X	BG$_{31}$		+		+		+	−	−
	BG$_{34}$						+		
XI	BG$_7$			+	+	−	−		
	BG$_{36}$							+	+
XII	BJ$_1$			+	+				+
	BJ$_2$					+	+	+	
	BJ$_5$	+		+	+	+	+	+	+
	BJ$_7$					+		+	
XIII	BJ$_{20}$					−	−		
XIV	BJ$_{16}$	+		+					
	BJ$_{17}$			+		±	−	+	+
	BJ$_{21}$	+	+		+				

According to Lewis (1954), different interactions among alleles lead to a great complexity of incompatible relationships. In the case of four alleles, considering only dominance and co-dominance, nine patterns of interaction are possible. When the interactions in pistil and pollen are different, 81 combinations are possible. To study the interactions among alleles in pollen and stigma it is necessary to make reciprocal crosses among known heterozygous and homozygous genotypes, as in the studies on *Brassica* carried out by Wallace (1979 a).

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