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Testing methods for producing self-pollinated fruits in ornamental peppers

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ABSTRACT

The pepper market in Brazil is experiencing major changes. There is a mounting interest in new types of peppers, including those of ornamental relevance. The use of protected cultivation is raising the demand for high-yielding hybrids, with higher commercial value, so as to turn this production system into a profitable investment. Hybrid seeds are obtained by crossing endogamic lines, which, in their turn, result from successive self-pollinations. The objective of this study was to compare two methods of protecting selfed flower buds from contamination with exogenous pollen in ornamental peppers. The experiment was carried out in a greenhouse, on a laboratory of the Universidade Federal da Paraíba. We took plants of 15 hybrids and their reciprocals to test two methods to protect selfed flower buds: 1= bud covering with aluminum foil; and 2= bud sealing with white glue. We carried out ten self-pollinations per hybrid, in pre-anthesis, for each method. We evaluated the average fruit set rate per method within each hybrid. The general fruit setting average in method 1 (16%) was lower than in method 2 (51%); with not a single hybrid in which fruit set rate of method 1 has exceeded that of method 2. A few hybrids showed up to 90% fruit set after self-pollination when flower buds were sealed with white glue (method 2), in contrast to a maximum of 40% fruit set when using method 1. The direction of the cross, direct or reciprocal, also affected fruit set rate, causing either reduction or increase, but with no effects of the selfed bud protecting method. Considering our results, we indicate the use of white glue to seal selfed flower buds to secure the success of controlled selfing in pepper.

Keywords: *Capsicum annuum*, breeding, cross pollination, advanced generations.

RESUMO

Comparação de métodos para a produção de frutos autofecundados em pimenteiras ornamentais

O mercado de pimenteiras no Brasil passa por grandes modificações, sendo crescente a exploração de novos tipos de pimentas, incluindo aquelas de interesse ornamental. Com o plantio em ambientes protegidos, aumentou a procura por híbridos com maior produtividade e valor comercial, de forma a tornar rentável o investimento nessa infraestrutura de produção. As sementes híbridas são obtidas a partir do cruzamento de linhagens endogâmicas que por sua vez, são obtidas por autofecundações sucessivas. O objetivo deste trabalho foi comparar dois métodos de proteção de botões florais após a autofecundação em pimenteiras ornamentais. O experimento foi conduzido em casa de vegetação, em laboratório da UFPB. Plantas de 15 híbridos e seus recíprocos foram utilizadas no teste de dois métodos de proteção do botão floral: 1= cobertura dos botões com papel alumínio e 2= fechamento dos botões com cola branca. Para cada metodologia foram realizadas dez autofecundações em cada híbrido, em pré-antese. Ao final, as médias de pegamento de frutos em cada metodologia foram contrastadas para cada híbrido. A média geral da taxa de pegamento de frutos observada para o método 1 (16%) foi inferior à observada para o método 2 (51%), não havendo um único híbrido em que a taxa de pegamento de frutos observada para o método 1 tenha superado aquela do método 2. Alguns híbridos apresentaram até 90% de pegamento de frutos quando se utilizou o método 2, em comparação a um máximo de 40% para o método 1. A direção do cruzamento, direto ou recíproco, também afetou a taxa de pegamento de frutos, porém sem influência do método de proteção do botão floral. Diante dos resultados encontrados neste trabalho, sugere-se o fechamento dos botões com cola branca para garantir o sucesso de autofecundações controladas em pimenteiras.

Palavras- chave: *Capsicum annuum*, melhoramento, fecundação cruzada, avanço de gerações.

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Peppers, which belong to the genus *Capsicum*, family Solanaceae, are native to America. Heiser (1995) states that *Capsicum* had between 25 and 30 species, five of them domesticated: *C. annuum*, *C. baccatum*, *C. frutescens*, *C. chinense*, and *C. pubescens*. More recently, Moscone *et al*. (2007) reported that the genus has already 31 identified species, thirteen occurring in Brazil, eleven wild. Pozzobon *et al*. (2006) karyotyped fourteen wild and semi-domesticated pepper species, three of them not yet botanically identified. Peppers bear a wide diversity, with a variety of fruit shapes, sizes, colors, flavors, pungency, and nutritional composition, and also with different plant architectures (Bosland & Votava, 1999). Pepper species and varieties are differentiated by botanical characteristics, mainly in flowers and fruits (Carvalho *et al*., 2003).

Pepper market in Brazil is currently experiencing major changes. New types of peppers are being explored, as well as products with high added value, such as canned peppers, exotic jams, chocolate with pepper, and other forms of processing. In this scenario, we should also include the use of peppers for ornamental purposes. Pepper cultivars developed to this aim must be easy to propagate and grow, with short vegetative phases and marked esthetic value. As few ornamental pepper cultivars are available in the Brazilian market, there is plenty of room and opportunities for breeding programs dedicated to this goal (Rufino & Hairstyle, 2006). The ornamental pepper seeds available today within the country are mostly hybrid, from cultivars Gion Red, *Pirâmide*, *Espaguetinho Ornamental*, and Grisu F_1 (Fabri, 2008).

At the Universidade Federal da Paraíba, we carry out an ornamental pepper breeding program, based on mass selection with progeny test, using both SSD (Single Seed Descent) and the genealogic method. The program develops hybrids and segregating populations, whose genetic studies generate useful information for the program itself, as well as to other breeding actions in pepper (Rego *et al*., 2011, 2012). We already reached several positive results, as the selection of lines with longer post-production phases (Rego *et al*., 2010), selection of ethylene resistant lines (Santos *et al*., 2012), and the development of 15 *Capsicum annuum* hybrids, currently under evaluation.

The knowledge of a given species reproductive biology is essential to develop breeding programs. Pepper flowers are hermaphrodite and self-compatible, which favors self-pollination. However, cross-pollination does occur at levels that vary between and within species from 0.5 to 70%. Therefore, *Capsicum* species are classified in an intermediate group between allogamous and autogamous. In domesticated species, stigma and anthers are on the same plane in the flower, permitting self pollination. On the other hand, on wild species the stigma is above anthers, facilitating cross-fertilization (Casali & Couto, 1984). Being acquainted with the flower reproductive traits is critical at all stages of breeding programs, due to the need of conducting segregating generations, as well as in the production of hybrid and genetic seeds. It is this knowledge that will shape the tools to avoid seed genetic contamination (Bosland 1993, 1996).

Although hybrid seeds come from cross-pollination, to develop hybrids is necessary first to produce endogamic lines, obtained out of successive self-pollinations. However, when natural selfing is subjected to cross fertilization rates with the range of those observed in *Capsicum*, the development of truly pure endogamic lines is jeopardized. In such cases, artificial selfing, followed by measures to prevent pollen contamination from occurring, is the way out.

The objective of this study was to compare two techniques to protect selfed flower buds on ornamental peppers following selfing.

MATERIAL AND METHODS

We carried out this work from March to December 2009, in greenhouse, in the laboratory of Plant Biotechnology of the Universidade Federal da Paraíba, located at the city of Areia (6°58'S, 35°41'W, 618 m over sea level) (Mascarenhas *et al*., 2005). As experimental material, we used fifteen hybrids and their reciprocals, obtained by crosses involving six *Capsicum annuum* accessions, in a diallel scheme. We tested two methods to protect flower buds after controlled self-pollination, 1= bud covering with aluminum foil (Rego, 2001) and; 2= bud sealing with ordinary white glue.

We sowed the F_1 seeds in 128-cell polystyrene trays and transplanted them to 900 mL-plastic pots when seedlings had six true leaves. Trays and pots were filled with commercial substrate (Plantmax®) and kept in greenhouse. As flower buds appeared, we manually selfed them in pre-anthesis, and protected the selfed buds using one of the two methods under evaluation. The bud cover made with aluminum foil was shaped using a pen, in sizes large enough to cover the whole bud. The white glue was applied to the buds using a spatula. For each method we performed at least ten selfings by hybrid, totaling more than 300 selfings per method. We scored the number of fruits set and calculated the fruit set percentage by hybrid and by bud protecting method.

We analyzed the means for fruit set for all hybrids (direct and reciprocal), as well as for each of the two methods under evaluation. Data were compared by the t test, at 1% probability, using the software GENES (Cruz, 2006). For performing the t test, we contrasted the means for fruit set for each method within hybrids, using the following expression:

$$t = \frac{Yi - Yi}{\sqrt{\frac{Vi}{ni} + \frac{Vj}{nj}}}$$

where Yi= mean for fruit set of a given hybrid using method 1 (bud covering with aluminum foil); Yj= means for fruit set of the same hybrid using method 2 (bud sealing with white glue); ni and nj= number of selfed buds used to obtain Yi and Yj and; Vi and Vj= variances associated to Yi and Yj, respectively.

RESULTS AND DISCUSSION

The general average of fruit set rate when selfed buds were protected using method 1 (16%), aluminum foil, was considerably lower than the general average for fruit set rate observed for method 2 (51%), white glue; with not a single hybrid in which the rate for method 1 has exceeded the rate for method 2 (Table 1). A few hybrids showed up to 90% fruit set when flower buds were sealed with white glue (method 2), in contrast to a maximum of 40% fruit set when using method 1 (Table 1).

Most pepper species show self-compatibility, except for *C. cardenasii* and *C. pubescens* (Rego *et al*., 2011); although in the latter there are also self-compatible varieties. Despite the widespread occurrence

Table 1. Fruit set after controlled self-pollination in *Capsicum annuum* hybrids (pegamento de frutos após autofecundações controladas em híbridos de *Capsicum annuum*). Areia, UFPB, 2010.

Hybrids	Protected flower buds (nº)		Harvested fruits (nº)		Fruit set rate (%)	
	Aluminum foil	White glue	Aluminum foil	White glue	Aluminum foil	White glue
77.1 x 01	20	10	4	4	20	40*
01 x 77.1	20	10	4	6	20	60*
132 x 01	10	10	2	5	20	50*
01 x 132	10	10	1	5	10	50*
77.2 x 01	10	20	2	10	20	50*
01 x 77.2	10	10	2	5	20	50*
134 x 01	10	10	1	6	10	60*
01 x 134	10	10	2	5	20	50*
137 x 01	10	20	1	4	10	20*
01 x 137	10	10	1	2	10	20*
77.2 x 77.1	10	10	2	4	20	40*
77.1 X 77.2	10	10	2	4	20	40*
132 x 77.1	20	10	0	3	0	30*
77.1 x 132	10	10	1	2	10	20*
134 x 77.1	10	10	3	7	30	70*
77.1 x 134	10	10	3	7	30	70*
137 x 77.1	20	10	0	2	0	20*
77.1 x 137	20	10	0	9	0	90*
132 x 77.2.	10	10	1	2	10	20*
77.2 x 132	10	10	2	5	20	50*
134 x 77.2	20	20	2	18	10	90*
77.2 x 134	10	10	3	8	30	80*
137 x 77.2	10	10	0	8	0	80*
77.2 x 137	10	10	1	3	10	30*
134 x 132	10	10	4	9	40	90*
132 x 134	10	10	1	5	10	50*
137 x 132	10	10	4	7	40	70*
132 x 137	10	10	1	5	10	50*
137 x 134	20	20	4	8	20	40*
134 x 137	10	10	1	4	10	40*
Average					16**	51**

*Fruit set rates in the same line differ significantly from each other, p>0.01; t test (taxas de pegamento de fruto na mesma linha diferem significativamente entre si, p>0,01; teste t).

of self-compatibility in *Capsicum*, the breeder must take measures to prevent spontaneous cross-pollination from taking place. Outcross rates in *Capsicum*, which is extremely influenced by the presence of pollinating insects, ranges from 2 to 90% (Bosland, 1993; Pickersgill, 1997). Such figures place *Capsicum* plants as facultative allogamous (Odland & Porter, 1941; Franceschetti, 1971; Tanksley, 1984).

Preventing spontaneous cross-pollination from occurring is a key requirement to secure the production of genuine hybrid and genetic seeds, as well as seeds in all other stages of the breeding program (Bosland 1993, 1996). Bosland (1993) suggests covering the plants with a thin cloth to circumvent cross-pollination. Rego (2001) covered plants of *C. baccatum* with organza, but noticed that fruit development was compromised in the plants that were covered. Considering this fact, along with the results of the current work, we suggest sealing flower buds with white glue after controlled selfing as an efficient method to avoid cross-fertilization in ornamental pepper.

The direction of the cross, direct or reciprocal, in the several crossing pairs tested in this work, also affected fruit set rate, causing either reduction or increase, but with no effects of the selfed bud protecting method. We observed influence of the direction of the cross on fruit set rate in 66 and 60% of the crossing pairs when methods 1 and 2 were used respectively (Table 1). For instance, accession 77.1 had 90% fruit when used as the female parent and merely 20% when used as the pollen source (Table 1). Nascimento *et al*. (2011) also found lower percentages of seed germination and vigor when accession 77.1 was used as male parent in crosses with accession 137, demonstrating that these characteristics are influenced by maternal effects.

Cruz & Campos (2007), comparing different pollination methods in *Capsicum frutescens*, reported that fruit set decreases with time. These authors found 57.1% fruit set straight after spontaneous selfing. However, the fruit persistence rate fell from 50 to 30% from 15 to 30 days after pollination and to only 20% in the end of the cycle. When the same authors used bee-mediated cross-pollination, the fruit set rate after selfing was 88.5%, with fruit persistence decreasing to 84.6, 78.8, and 59.6%, 15 and 30 days after pollination and at harvest, respectively. The *C. annuum* genotypes used in the current work may have behaved similarly, since fruit set rates we refer to in this study correspond to fruits harvested.

Cruz & Campos' (2007) results confirmed the need to prevent cross pollination from occurring in *Capsicum* and support the results reported by Bosland (1993, 1996), Rego (2001), and Rego *et al*. (2011). This set of papers also stresses the importance of performing controlled self-pollination to develop and maintain *Capsicum* inbred lines in breeding programs. Both methods to protect selfed flower buds used in this work were effective in allowing fruit set. Nevertheless, method 2, sealing flower buds with white glue, was more appropriate. Using method 2, it was possible to harvest fruits from all crosses performed. Thus, we recommend sealing flower buds of *Capsicum annuum* plants with white glue to produce fruits out of controlled self-pollination in ornamental peppers.

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