horticultura brasileira

Scientific communication

CRISPIM, JG; RÊGO, ER; RÊGO, MM; NASCIMENTO, NFF; BARROSO, PA. 2017. Stigma receptivity and anther dehiscence in ornamental pepper. *Horticultura Brasileira* 35: 609-612. DOI - http://dx.doi.org/10.1590/S0102-053620170421

Stigma receptivity and anther dehiscence in ornamental pepper

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ABSTRACT

The objective of this study was to evaluate stigma receptivity and anther dehiscence in three floral development stages in F_3 population of ornamental pepper (*Capsicum annuum*). A total of 162 plants (81 of purple corolla and 81 of white corolla) were used in this study. To analyze the stigma receptivity and anther dehiscence, three floral stages were analyzed: bud, pre-anthesis, and open flower. For each stage, 33 flowers of each phenotypic class were collected. The receptivity of the stigma was detected by dripping hydrogen peroxide solution (3%). For the evaluation of the level of receptivity, scores of 0 to 3 were assigned, varying according to the bubbles percentage found on the stigmatic surface. Scores of 1 to 5 were assigned for the reference of the amount of pollen released by the stamens. The data were subjected to analysis of variance ($p \leq 0.05$) following a 2x3 factorial scheme: two classes of flower colors and three development stages, with 33 replicates (flowers) with later separation of means by Tukey's test at the same probability level. For the analysis of divergence, the data were clustered according to Tocher's method based on generalized Mahalanobis distance. Despite the not significant differences between the means for pollen dehiscence of both flower colors, the purple flowers presented higher stigma receptivity than the white ones. The ornamental pepper flowers displayed stigma receptivity since the bud phase, but the highest level of receptivity was observed after anthesis. Anther dehiscence prevailed after anthesis of the flowers regardless of color, and the presence of pollen grains was superior after anthesis. For the manual crossing, the buds at pre-anthesis phase are the most indicated irrespective of the color, as they show high stigma receptivity and greater ease at handling.

Keywords: *Capsicum annuum*, breeding, floral biology, pollination.

RESUMO

Receptividade estigmática e deiscência das anteras em pimenteiras ornamentais

O objetivo deste trabalho foi avaliar a receptividade estigmática e a deiscência das anteras em três estádios de desenvolvimento floral, em uma geração F_3 de pimenteiras ornamentais (*Capsicum annuum*). Foram avaliadas 162 plantas divididas em duas classes (81 de corola roxa e 81 com corola branca). Para estudar a receptividade dos estigmas e a deiscência das anteras foram considerados três estádios florais: botão, pré-antese e flor aberta. Para cada estádio foram coletadas 33 flores de cada classe fenotípica. A receptividade do estigma foi constatada por meio do gotejamento de uma solução de peróxido de hidrogênio (3%) sobre o mesmo. Para a avaliação do nível de receptividade foram atribuídas notas de 0 a 3 variando de acordo com porcentagem de bolhas encontradas na superfície estigmática. Para referência da quantidade de pólen liberado pelos estames, foram atribuídas notas de 1 a 5. Os dados foram submetidos à análise de variância ($p \leq 0,05$), seguindo o esquema fatorial 2x3: duas classes de cor de flores e três estádios de desenvolvimento, com 33 flores como repetições, com posterior separação das médias pelo teste de Tukey neste mesmo nível de probabilidade. Para análise de divergência os dados foram agrupados de acordo com o método de Tocher baseado na distância generalizada de Mahalanobis. Após a análise dos dados observou-se que as cores da flor, não diferiram estatisticamente, para a presença da deiscência das anteras, entretanto, as flores de cor roxa apresentaram maior receptividade no estigma. As flores das pimenteiras ornamentais apresentam receptividade estigmática desde a fase de botão, entretanto o maior nível de receptividade foi observado após a antese. A deiscência das anteras predominou após a antese das flores independente da cor, sendo a presença de grãos de pólen superior após a antese. Para a realização de cruzamento manual, os botões na pré-antese são os mais indicados independente da cor, pois apresentam alta receptividade estigmática e maior facilidade de manuseio.

Palavras-chave: *Capsicum annuum*, melhoramento, biologia floral, polinização.

Received on May 24, 2016; accepted on May 2, 2017

Pepper is an important spice; a symbol of the world culinary that has gained prominence as functional food because of the high nutritional value of its fruits, rich in vitamins A, C and E, carotenes, essential minerals, and antioxidant substances (Rêgo *et al.*, 2012a). In addition to being consumed in their natural state, peppers can be processed and used in several lines of products in the food industry, e.g., in making of condiments and sauces, besides medicinal and, more recently, ornamental uses (Ohara & Pinto, 2012; Rêgo *et al.*, 2012b).

In Brazil, the cultivation and sale of ornamental peppers have grown in the last few years (Nascimento *et al*., 2011). Because of their economic importance, the demand for the breeding of peppers and release of new cultivars is constant. Not every pepper cultivar adapts to cultivation in a pot, with variations present even within the same species. Only those which show reduced plant size and harmony in the pot can be grown and marketed as ornamental plants. The traits plant height, total height (heights of plant and pot), canopy width, color and position of the fruit and flower are criteria utilized by consumers at the moment of purchase (Barroso *et al*., 2012; Nascimento *et al.,* 2013; Rêgo *et al*., 2015).

The artificial crossings are based on the selection of lines, and from the latter, the development of hybrid cultivars that combine desired traits from their parents. To perform crossing with good fruit set, it is essential to understand the reproductive biology of the plant. Among these aspects are the pollen viability and stigma receptivity, because success in artificial crossings requires viable pollen and a receptive stigma at the moment of crossing (Antonio, 2004). In controlled crossings, knowing the floral stage that the stigma is receptive, may determine the success or failure of artificial pollination.

Hybridization within pepper species, involving different types or cultivars, has not been explored much (Rêgo *et al*., 2009). According to Rêgo *et al*. (2012b), among the factors contributing to the restriction of the use of hybridization in the breeding of *Capsicum*, are the low pollen viability, the difficulty to handle flowers and low yield of seeds per fruit. A stigma is characterized as receptive when it is able to receive the pollen grain and promote its germination. The floral stage that characterizes stigma receptivity varies from species to species. The stigma surface and its components have a crucial role in the pollen adhesion processes, hydration, germination, and growth of the pollen tube inside the stigma. Thus, the presence of esterases and peroxidases on the stigmatic surface has been considered indicative for receptivity (Dafni & Maués, 1998).

Information related to aspects of the reproductive biology of peppers such as receptivity and anther dehiscence are of great importance for planning and performing the breeding of this culture, as well as for defining the feasibility of the strategies to be adopted. Thus, the objective of this study was to evaluate the stigma receptivity and anther dehiscence in three floral development stages, determining which of these best characterizes the receptivity phase of the stigma and dehiscence of pollen in a F_3 generation of ornamental pepper (*Capsicum annuum*).

MATERIAL AND METHODS

The study was conducted on the experimental field of the Laboratório de Biotecnologia Vegetal do Centro de Ciências Agrárias (CCA) at Universidade Federal da Paraíba (UFPB), located in the city of Areia-PB, Brazil (6°58'S, 35°41'W, 618 m altitude). F_3 genotypes, 162 individual plants, of ornamental pepper were sown in trays with 200-cell capacity and after having six true leaves they were transplanted to the field. These individual plants were obtained by the self-fertilization of an F_2 population. This F_2 population was the result of a self-fertilization of an F_1, resultant of the cross between the accessions UFPB 134 and UFPB 77.2. Both parents belong to Germplasm Bank of CCA-UFPB.

The cultural management was carried out according to Filgueira (2009). A total of 162 plants: 81 of purple corolla and 81 of white corolla were used in this study. To analyze the stigma receptivity and pollen dehiscence, three floral stages were used: bud, pre-anthesis, and open flower. For each stage, 33 flowers of each phenotypic class were collected.

To study the stigma receptivity, the following three floral development stages were considered: bud (stage I), pre-anthesis (stage II), and open flower (stage III). Thirty-three flowers were evaluated of each phenotypic class for each development stage. The material was collected and immediately taken to the laboratory, where observations were made with a stereoscopic microscope. The receptivity of the stigma was detected by dripping a drop of hydrogen peroxide solution (3%) on it. To evaluate the level of receptivity, scores from 0 to 3 were assigned according to the amount of bubbles found on the stigmatic surface: 0% (0), 30% (1), 60% (2), and 100% (3). The analyzed variables were anther dehiscence and stigma receptivity. Scores from 1 to 5 were assigned for the reference of the amount of pollen liberated by the stamens, as follows: 0%, anther without pollen (1); 40%, base covered with pollen (2); 60%, anther with pollen up to the middle (3); 80%, only the apical tip without pollen (4); and 100%, anthers entirely covered with pollen (5). The data were subjected to analysis of variance ($p \leq 0.05$) following a 2x3 factorial scheme: two classes of flower colors and three development stages, with 33 replicates (flowers) with later separation of means by Tukey's test at the same probability level. With the goal of grouping the six evaluated treatments considering all evaluated variables the data were subjected to analysis of divergence based on generalized Mahalanobis distance with cluster using Tocher's method.

RESULTS AND DISCUSSION

There was no significant interaction between the flower colors and development stage. For anther dehiscence, there was significant difference to development stages but not for flower colors. For stigma receptivity there were significant differences for both evaluated variables ($p \leq 0.05$) (Table 1). The coefficients of variation (CV) of the experiment were 22.85% for anther dehiscence and 38.80% for stigma receptivity. The coefficient of variation gives an idea of precision of the experiment. According to Silva *et al*. (2011), the classification of coefficient of variations of morphological variables of *Capsicum* peppers depends on the studied variable.

Despite the not significant differences between the means for pollen dehiscence of both flower colors, the

Table 1. Summary of analysis of variance of floral biology traits in ornamental peppers (*Capsicum. annuum*). Areia, UFPB, 2014.

Source of variation	Mean square	
	Anther dehiscence	Stigma receptivity
Flower stage (S)	273.065 **	13.883 **
Flower color (C)	0.409 ns	4.545 **
S x C	0.469 ns	0.045 ns
CV (%)	22.85	38.80

**Significant and nsnot significant by F test (p≤0.05).

Table 2. Means for pollen dehiscence and stigma receptivity of different flower colors of ornamental peppers (*Capsicum annuum*). Areia, UFPB, 2014.

Flower color	Anther dehiscence	Stigma receptivity
White	2.292 a	1.737 b
Purple	2.383 a	2.04 a

To evaluate anther dehiscence, scores from 1 to 5 were assigned indicating the amount of pollen liberated by the stamens, as follows: 1= anther without pollen (0%); 2= base covered with pollen (40%); 3= anther with pollen up to the middle (60%); 4= only the apical tip without pollen (80%); 5= anthers entirely covered with pollen (100%). To evaluate the level of stigma receptivity, scores from 0 to 3 were assigned, as follows: 0= 0%; 1= 30%; 2= 60%; 3= 100%. *Means followed by the same letter on the column, do not differ based on Tukey's test (p≤0.05).

Table 3. Means for pollen dehiscence and stigma receptivity of different flower stages of ornamental peppers (*Capsicum. annuum*). Areia, UFPB, 2014.

Flower stage	Anther dehiscence	Stigma receptivity
Flower	4.689 a	2.378 a
Pre-anthesis 'balloon stage'	1.303 b	1.818 b
Bud	1.03 c	1.469 c

To evaluate anther dehiscence, scores from 1 to 5 were assigned indicating the amount of pollen liberated by the stamens, as follows: 1= anther without pollen (0%); 2= base covered with pollen (40%); 3= anther with pollen up to the middle (60%); 4= only the apical tip without pollen (80%); 5= anthers entirely covered with pollen (100%). To evaluate the level of stigma receptivity, scores from 0 to 3 were assigned, as follows: 0= 0%; 1= 30%; 2= 60%; 3= 100%. *Means followed by the same letter on the column, do not differ based on Tukey's test (p≤0.05).

purple flowers presented higher stigma receptivity than the white ones (Table 2). Cruz & Campos (2007), studying anther dehiscence in *C. frutescens*, found similar results, with stigma receptivity occurring approximately one hour after anthesis. However, flowers that opened in the afternoon, after 16:00 hours, presented anther dehiscence only on the morning of the following day. According to Aleemullah *et al*. (2000), the dehiscence pattern of anthers suggests that this event is partially controlled by endogenous rhythms of the flower. Cruz & Campos (2007) showed the high level of stigma receptivity occurring at anthesis in *Capsicum frutescens* species.

The flowering phase was superior when compared with the other development stages, evaluated for both anther dehiscence and stigma receptivity (Table 3). According to Silva *et al*. (2005), the stigma are more viscous at the anthesis stage. This fact favors the pollen adherence and the fertilization. Brito *et al*. (2015), working with flower buds of *Nicotiana tabacum*, showed that some other morphological and/or physiological factors, may limit the number of pollen tubes growing in pistils of younger stages. This fact can also be observed in flowers of *Capsicum annuum*. Cruz & Campos (2007) found similar data in malagueta chili pepper (*Capsicum frutescens*).

The obtained results are similar to those found by Free (1993) in a study on the stigma receptivity in pepper flowers, wherein the author observed that the flowers had a receptive stigma before anther dehiscence, indicating that buds at pre-anthesis (stage II) can be used for manual crossing to avoid pollen contamination. In this stage the stigmas are receptive, but cannot self-pollinate, as the pollen grains are not available yet. In this stage, besides the increased receptivity, handling is also easier when compared to the bud stage (stage I), thereby reducing the mechanical shocks that may lead to style breaking, which would prevent pollination.

The use of Tocher's optimization method based on generalized Mahalanobis distance formed three groups. The first consisted of the white and purple buds in the balloon stage and by the purple buds; the second clustered the purple and white flowers; and the third and last group was comprised of the white bud only, which, for the stigma receptivity, was the one that showed the lowest receptivity. In addition to the difficulty of handling, the bud phase (stage I) should present a low fruit set even when pollinated by viable pollen grains because stigma receptivity is low.

Based on the results, it can be affirmed that anther dehiscence predominated after the anthesis of the flowers regardless of color. However, pepper flowers show stigma receptivity at bud stage, and the greatest level of receptivity is observed after anthesis.

For manual crossing, the buds pre-anthesis is the most indicated regardless of color, as the flowers have high stigma receptivity and facilitate handling.

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