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Meiotic behavior and pollinic viability in bean cultivars

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Key words: Acetic orcein, *Phaseolus vulgaris,* pollen, Alexander's reactive.

ABSTRACT: The aims of this study were to determine the meiotic behavior and to estimate pollen grains viability in bean (*Phaseolus vulgaris* L.) cultivars. Flower buds were collected during different developmental stages of the Mesoamerican bean cultivars IAPAR 44, Guapo Brilhante, BRS Expedito, BRS Valente, Guateian 6662 and Pérola, and the Andean bean cultivar Iraí, grown in a greenhouse. The meiotic index was determined by anther squashing of material fixed in absolute ethanol-glacial acetic acid (3:1) and stained with acetic orcein. No meiotic abnormalities were observed and the meiotic indices were high for all cultivars, indicating that the mismatch generated during crosses is not related to any meiotic changes. Estimation of pollen viability was made by comparing acetic orcein staining vs. Alexander's reactive: pollen viability was high in all cultivars with either stain, but was significantly higher when using the acetic orcein stain (>99%). Though some cultivar showed a significantly smaller pollen size, the range of variation among cultivars was low (means' range 51-66 µm).

Introduction

The common bean (*Phaseolus vulgaris* L.) is an annual, predominantly autogamous legume, domesticated for more than 7000 years in two presumptively original centers: Mesoamerica (Mexico and Central America) and the Andean region. It is believed that beans, along with corn and pumpkin, began as a weed in the cultivation of cassava and sweet potatoes in Central America. For millenia, farmers cultivated complex mixtures of types of beans as a hedge against drought, diseases and pest attacks (Gepts and Debouck 1991). This process produced a nearly unlimited genetic variability with a wide variety of colors, texture and grain size, meeting with the growing conditions and taste preferences of different regions (Schoonhoven and Voysest 1991).

The Andean set is characterized primarily by large bean grains and phaseolin T, C, H and A, while the Mesoamerican has small seeds and phaseolin S (Gepts and Debouck 1991). Moreover, these sets differ in various other morphological characteristics and resistance to pathogens, which stimulated the interest of breeders in the use of inter-gene pool hybrids. However, the use of a higher genetic divergence by crossing between Andean and Mesoamerican cultivars, in many situations, is not possible due to genetic incompatibility manifested in the F1 generation plants (Vieira *et al.* 2005). This mismatch manifests itself in the form of dwarfism or weakness of the F1 hybrids, whose plants die or have weak growth, showing several changes, such as sterility, reduced root growth, chlorotic leaves, no roots, and formation of adventitious roots in the hypocotyl region, among other abnormalities (Vieira *et al.* 2005, Vilarinho 2004).

Knowledge generated by cytogenetics is important for breeding programs, as it helps to select appropriate materials and maintain constant monitoring, revealing changes in the fertility of individuals (Navarini 2008). According to Sy-

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benga (1998), cytogenetics has two main functions in plant breeding: generate information and provide methods for genetic manipulations. Considering that it is during meiosis that gene recombination occurs and gametogenesis events are controlled by a large number of genes, meiosis is considered a source of genetic variability which the organisms possess in order to adapt to the environment in which they live and thus ensure their perpetuation through descent (Golvbovskaya 1979, Sing, 1993, Nassar 1997, Pagliarini 2000).

The aim of plant breeding programs is to achieve better cultivars, from genetic manipulation of the existing germplasm of certain species. Selection of genotypes and crossings are among the most important factors for the success of these programs. The effectiveness of crossings, both among cultivars of a species and between species, depends directly on pollen viability (Techio *et al.* 2006).

Chromosomal irregularities directly reflect on the reproductive processes of the species by forming nonviable pollen grains (Marutani *et al.* 1993, Corrêa *et al.* 2005). Gametes carrying abnormalities are less competitive than normal gametes, because they reduce the formation of fruit with or without seeds (Pagliarini 2001).

Pollen grain behavior in any plant species is fundamentally important for the study and genetic detailing of the plant, in order to study the reproductive behavior and to better understand the dispersal of male gametes (Corrêa *et al.* 2005), as well as for the practical application in conservation, breeding, or agriculture cultivation (Guinet 1989). Besides that, it is an important factor in distinguishing species (Karsburg and Battistin 2005).

Pollen viability is an important parameter in plant studies, indicating the male reproductive potential of the species, and also contributing in taxonomic, ecological, and palynological, studies, which provide basic practical information for genetic conservation, as well as in agriculture, for the planning of improvement and cultivation (Alexander 1980).

Considering that the manifestation of the genotype of an individual is the result of the contribution brought by male and female gametes, the higher the pollen viability, the greater the possibility of forming different combinations of alleles and ultimately, genetic variability (Souza *et al.* 2002).

Pollen viability can be determined by direct methods, such as induction of pollen germination *in vivo* or *in vitro* and indirect methods, based on cytological parameters such as staining (Shivanna and Johri 1985, Dafni 1992, Shivanna and Rangaswamy 1992, Kearns and Inouye 1993). The aim of this study was to determine the meiotic behavior and viability of pollen grains in bean cultivars (*Phaseolus vulgaris* L.) by comparing two staining methods.

Materials and Methods

The plant material used were flower buds from Mesoamerican bean cultivars IAPAR 44, Guapo Brilhante, BRS Expedito, BRS Valente, Guateian 6662 and Pérola and from the Andean bean cultivar Iraí, sown in plastic pots with a capacity of 5 L, containing a mixture of soil + substrate Plantmax®, 2:1 (v/v). The soil used was typical alithic Bruno-Grey Argisol, with the following chemical composition: pH

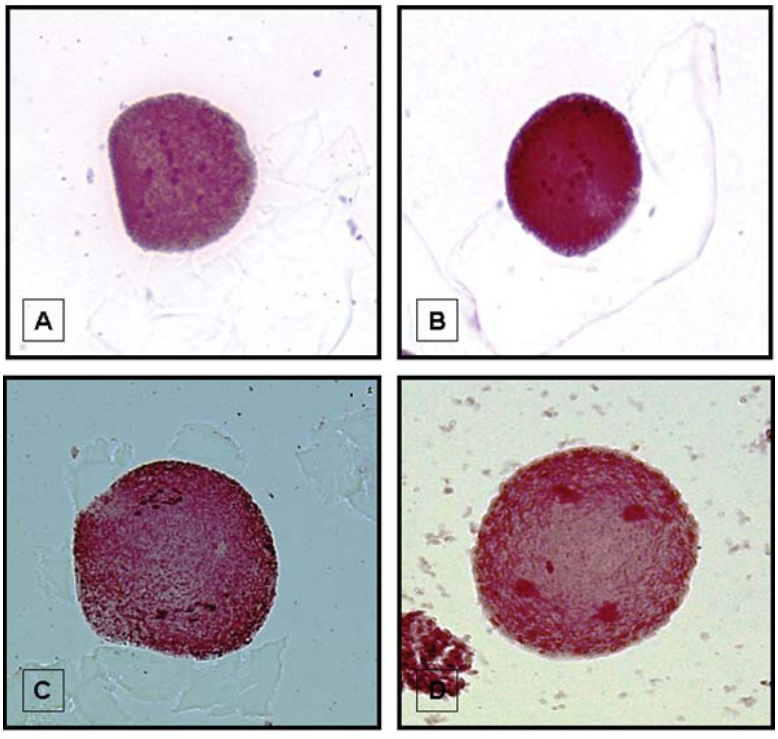

FIGURE 1. A and B, diakinesis; C, anaphase I; D, telophase II

5.8; organic matter: 1.9%; phosphorus: 15.3 mg dm^{-3}; potassium: 84 mg dm^{-3}; calcium: 5.8 cmolc dm^{-3}; magnesium: 2.4 $cmol_c$ dm^{-3}; sulfur: 11.6 mg dm^{-3}. The correlation of soil fertility was performed for the minerals considered limited in the soil. In each pot two plants were throughout the end of the end of their cycle with a total of eight plants of each parent, sown on 04 August 2010 and cultivated in a greenhouse.

During the blooming period (October 2010), floral buds were collected between 08:00-11:00, in the R5 reproductive stage (CEPEF, 2007), and fixed in absolute ethanol-glacial acetic acid (3:1) for 24-72 h, at room temperature. Afterwards, the floral buds were transferred into 70% ethanol and maintained refrigerated (15ºC) until use.

For the study of meiosis, the slides were prepared by squashing the anthers (Guerra and Souza 2002), using acetic orcein 2% for cell staining. Two slides per each plant were prepared, eight plants for each cultivar. In the associations of the chromosomes in diakinesis and metaphase I, normal cells were considered those that presented 11 bivalents and in the disjunction of chromosomes in anaphase and telophase I, those with regular separation of 11 duplicated chromosomes at each pole. Chromosome disjunction was also analyzed in anaphase and telophase II, whereas normal cells were those that presented a distribution of 11 simple chromosomes for each pole (Tedesco 2000). The meiotic index (MI) was calculated according to Love (1949) MI=number of normal tetrads / number of total tetrads * 100.

The pollen mother cells (PMC) were evaluated on the number and viability of microspores in each cultivar. The PMC with four viable microspores were considered normal tetrads, those that presented less than four microspores (dyads and triads), more than four (polyads) or tetrads with sterile microspores (not stained) were considered abnormal.

To undertake the estimation of pollen grain viability, slides were prepared by the squashing technique of the anthers (Guerra and Souza 2002). Two slides were prepared for each plant, counting 800 pollen grains per plant, obtained from two plants per pot, being four pots per cultivar. Two types of stains were used, acetic orcein 2% and Alexander's reactive (malachite-green + acid fuchsin) (Alexander 1980).

For the estimation of pollen grain viability, pollen grains were considered unviable when they had abnormal size, weak staining, reduced and/or absent protoplasm, and were considered viable those that had an intact exine, with a well-stained, homogenously distributed protoplasm (Tedesco 2000). Furthermore, 100 mature pollen grains of each cultivar were measured to verify significant differences that lead to pollen non-viability, and, consequently, incompatibility in crosses.

The experimental design was completely randomized, with eight replications. The treatments were arranged in a bifactorial scheme 7x2, with seven cultivars (IAPAR 44, Guapo Brilhante, BRS Expedito, BRS Valente, Guateian 6662, Pérola, and Iraí) and two stains tested (acetic orcein 2% & and Alexander's reactive). The experimental unit consisted of two slides per stain in which 400 pollen grains per slide were scored. Each treatment was repeated 4 times, for a total of 1600 pollen grains per replicate. The results were compared by ANOVA, followed by the Tukey test (α=0.05), using the statistical program Assistat.

Results and Discussion

The results on meiotic behavior are reported in Table 1. No abnormal cells were observed (Fig. 1 A-D). Pairing between bivalents was normal during metaphase I, resulting in regular chromosomal segregation during anaphase I and anaphase II, without forming anaphasic bridges. At the end of sporogenesis, four microspores (tetrads) were formed

TABLE 1

Biological variables measured at 135 min in the perfusate (n = 10 in each group)

	Meiosis I			Meiosis II			
Cultivars	**Diakinesis**	**Metaphase I**	**Anaphase I**	**Telophase I**	**Metaphase II**	**Anaphase II**	**Telophase II**
IAPAR 44	44	20	11	41	05	04	42
Guapo Brilhante	28	12	08	28	08	05	30
BRS Expedito	44	11	10	06	04	05	13
BRS Valente	31	11	04	26	05	03	37
Guateian 6662	36	16	02	45	10	06	41
Pérola	42	06	13	05	03	08	16
Iraí	11	06	09	52	03	04	56

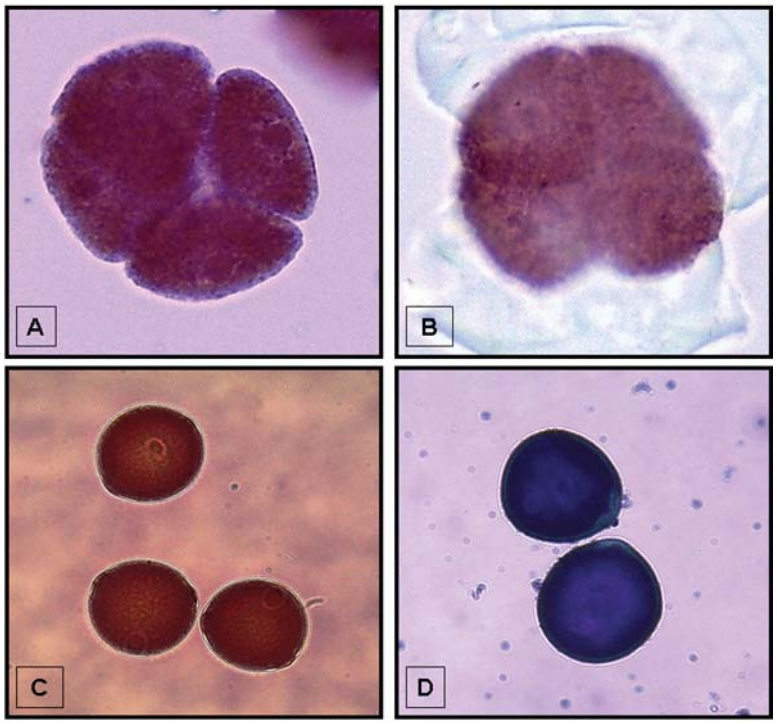

FIGURE 2. A and B, tetrads; C, pollen grains stained with acetic orcein; D, pollen grains stained with Alexander's reactive.

in most of the evaluated accessions, revealing a completely regular meiosis in terms of cell division and therefore with the formation of viable pollen grains.

When analyzing the MI of bean cultivars, triads, dyads, and polyads between mother cells and abnormal pollen were observed. Table 2 shows that the studied cultivars did not have significant differences between them, with $\chi^2 = 6.086$ and $p = 0.4136$ showing that a high percentage of viable pollen grains is expected as a result of a high percentage of normal tetrads (Fig. 2, A and B), which reflect directly on the uniformity between the cultivars on the regular meiotic process. The high meiotic indices, including the Andean cultivar Iraí, show that the incompatibility generated in the crossings is not related to some meiotic alteration.

Table 3 shows the results of comparing pollen grain viability estimates the two different stains for the seven cultivars, which were generally high. We observed that acetic orcein presented the highest viability in the majority of the cultivars, while Alexander's reactive presented a lower and/or similar viability percentage to that of acetic orcein.

For acetic orcein, the cultivar IAPAR 44 differed significantly from BRS Valente and from Iraí, not differing from the other cultivars. While the cultivars Guapo Brilhante, BRS Expedito, Guateian 6662, and Pérola did not have significant differences among each other.

When comparing the means for Alexander's reactive, the cultivars did not differ among each other. Guapo Brilhante presented significant differences when comparing

TABLE 2

Meiotic index (MI) in different bean cultivars (acetic orcein)

Cultivars	Number of tetrads	Number of triads	Number of dyads	Number of polyads	MI
IAPAR 44	487	4	2	10	96.8%
Guapo Brilhante	304	5	2	8	95.3%
BRS Expedito	270	4	1	10	94.7%
BRS Valente	300	1	2	6	97.1%
Guateian 6662	320	9	1	9	94.4%
Pérola	357	3	4	7	96.2%
Iraí	289	4	2	10	94.8%

TABLE 3

Pollen viability estimates (%) in different cultivars with two staining methods

	Acetic orcein 2%		Alexander's reactive	
Cultivars	**N***	**%**	**N**	**%**
IAPAR 44	6352	99.2^{bA}	6145	10
Guapo Brilhante	6379	99.7 ^{abA}	6348	8
BRS Expedito	6373	99.6 ^{abA}	6258	10
BRS Valente	6387	99.8 ^{aA}	6261	6
Guateian 6662	6367	99.5^{abA}	6351	9
Pérola	6381	99.7^{abA}	6363	7
Iraí	6391	99.88^{aA}	6371	10
Total	44630	99.61^{A}	44097	

* Means followed by the same lowercase letter in the column and uppercase in the line don't differ between each other by the Tukey test at 5% probability.

acetic orcein (99.67%) and Alexander's reactive (99.18%). Techio et al. (2006) using accessions of elephant grass and millet showed high pollen grain viability (higher than 90%), independent of the stain used. The elevated percent of functional pollen in these accessions is associated to meiotic regularity (Techio *et al.* 2006), which contributes to the use of these species in plant breeding programs.

Table 3 also shows that acetic orcein (99.61%) differed significantly from Alexander's reactive (98.42%). According to Alexander (1980), his staining procedure may provide more accurate data on pollen viability, since a differential coloration of viable and unviable pollens is obtained (Fig. 2 D), due to the simultaneous use of green malachite and acid fuchsin. The first has an affinity to cellulose in the cell wall, staining it green, while fuchsin acid stains the protoplasm. In this way, since aborted pollen grains don't contain protoplasm, they are stained green.

Thus, it appears that acetic orcein slightly overestimates viability, since it may stain viable (Fig. 2 C) and unviable pollens with the same intensity. As there is no other comparative parameter, sometimes the distinction between viable and unviable pollen may be doubtful.

Table 4 presents the mean of pollen grain measurements for the analyzed cultivars. Comparing the means of the measurements of all the cultivars, we found that the cultivar BRS Valente had a different mean that those of IAPAR 44 and BRS Expedito. Pollen grain size did not differ statistically in the other cases, indicating that the size of pollen will have no influence if crosses between these cultivars are made.

TABLE 4

Pollen grain size in different cultivars

Cultivars	**Means (µm)***
IAPAR 44	66.2^{a}2±1.58 ^{a}
Guapo Brilhante	57.2^{ab}2±1.40 ^{ab}
BRS Expedito	62.5^{a}5±1.66 ^{a}
BRS Valente	51.3^{b}3±1.47 ^{b}
Guateian 6662	61.5^{ab}5± 1,53 ^{ab}
Pérola	60.0^{ab}0±1.82 ^{ab}
Iraí	57.8^{ab}8± 2,63 ^{ab}

* Means followed by the same lowercase letter in the column and uppercase in the line don't differ between each other by the Tukey test at 5% probability.

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