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Morphology and viability of castor bean genotypes pollen grains

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ABSTRACT. The objective of this work was to characterize the morphology and viability of the pollen of 15 genotypes of castor bean (*Ricinus communis* L.) and to generate information that can assist in the selection of highly promising male parents for future use in genetic improvement programs aimed at producing seeds for oil extraction. Acetolysis and scanning electron microscopy was used to characterize the morphology of the pollen. The viability of the pollen grains was estimated by *in vitro* germination and colorimetric analysis (acetocarmine 2% and 2, 3, 5-triphenyltetrazolium chloride 1%). For the *in vitro* germination, pollen grains were grown in 10 types of solidified culture medium consisting of different concentrations of sucrose, boric acid, calcium nitrate, magnesium sulfate and potassium nitrate. The pollen grains had the following characteristics: medium size, isopolar and subspheroidal shape, radial symmetry, circular ambit, 3-colporate, elongated endoapertures, tectate exine and granulated sexine. The acetocarmine dye overestimated pollen viability. The media M5 and M8 were the most efficient at promoting the germination of pollen grains. The studied genotypes had high levels of viability and can therefore be used as male parents in genetic improvement programs.

Keywords: *Ricinus communis* L., *in vitro* germination, colorimetric analysis, pollen tube.

Morfologia e viabilidade de grãos de pólen de genótipos de mamoneira

RESUMO. O objetivo deste trabalho foi caracterizar a morfologia e a viabilidade polínica em quinze genótipos de mamoneira (*Ricinus communis* L.), a fim de gerar informações que possam auxiliar a seleção de genitores masculinos altamente promissores para posterior utilização em programas de melhoramento, tendo em vista a produção de sementes para a extração de óleos. As descrições morfopolínicas foram feitas a partir da acetólise e microscopia eletrônica de varredura. A viabilidade dos grãos de pólen foi estimada por meio da germinação *in vitro* e análise colorimétrica (carmim acético 2% e 2, 3, 5-cloreto de trifeniltetrazólio 1%). Para a germinação *in vitro*, grãos de pólen foram inoculados em dez tipos de meio de cultura, constituídos de diferentes concentrações de sacarose, ácido bórico, nitrato de cálcio, sulfato de magnésio e nitrato de potássio e solidificado. Os genótipos apresentam grão de pólen de tamanho médio, isopolares, subesferoidal, simetria radial, âmbito circular, 3-colporados, endoaberturas lalongadas, exina tectada e sexina granulada. O corante carmim acético superestima a viabilidade de pólen. Os meios M5 e M8 foram os mais eficientes na germinação dos grãos de pólen. Os genótipos estudados podem ser utilizados como parental masculino em programas de melhoramento, já que apresentam altos índices de viabilidade.

Palavras-chave: *Ricinus communis* L., germinação *in vitro*, análise colorimétrica, tubo polínico.

Introduction

With growing demand for renewable and cleaner energy sources to produce oil for biodiesel, the castor bean (*Ricinus communis* L.) is being considered as a good option for farmers.

Plant-breeding techniques can be used to obtain new, more productive varieties that often differ in seasonality; these plants are of great value in selective breeding programs, as they can enable the storage, transportation and maintenance of pollen grains with high viability (Vargas, Souza, Silva, & Bobrowski, 2009).

Studies of pollen morphology, ultrastructure and viability are of great value to genetic improvement programs seeking to obtain potentially promising selections (Chagas, Pio, Chagas, Pasqual, & Bettiol Neto, 2010). The viability of pollen grains can be determined by direct methods such as the induction of *in vitro* (Dane & Ekici, 2011; Cuchiara, Silva, & Brobowski, 2012; Imani, Kazem, Saeed, & Seiyed, 2011; Machado et al., 2014) and *in vivo* (Dane & Ekici, 2011; Fakhim, Hajilou, & Zaare, 2011) germination or indirect methods based on cytological parameters such as staining (Ćalić,

Devrnja, Kostić, & Kostić, 2013; Munhoz, Luz, Meissner Filho, Barth, & Reinert, 2008).

The *in vitro* germination of pollen grains is the most commonly used viability assay in genetic improvement programs, as it simulates the style-stigma interaction, induces the growth of pollen tubes (Soares, Jesus, Souza, Santos-Serejo, & Oliveira, 2013), promotes fertilization and allows crossings between high quality genotypes that flower at different times. This technique has been widely studied in several species (Cuchiara et al., 2012; Machado et al., 2014), including oleaginous species such as the castor bean, cotton, soy, canola (rapeseed), oil palm, sunflower, babassu palm and peanuts. Each species requires a specific protocol and culture medium to obtain good germination. The basic medium used for *in vitro* assays consists of sucrose, boric acid and a variety of other substances (Zambon, Silva, Pio, Figueiredo, & Silva, 2014).

Colorimetric assays are extensively used to monitor the viability of pollen grains, as these assays are simple and faster than direct methods. However, they may overestimate viability, as non-viable grains can be stained due to the presence of enzymes, starch or other substances (Galletta, 1983). Acetocarmine, aniline blue, blue cotton, potassium iodide and 2, 3, 5-triphenyltetrazolium chloride are the most commonly used dyes for these assays; they differentially stain pollen grains, thus providing quick and cost-effective results.

Given this context, the objective of this study was to characterize the morphology and to investigate the viability of pollen from fifteen castor bean genotypes and to generate information that can assist in the selection of highly promising male parents for future use in genetic improvement programs aimed at producing seeds for oil extraction.

Material and methods

Pollen grains from male flowers were collected at anthesis from fifteen castor bean genotypes developed by the 'Empresa Baiana de Desenvolvimento Agrícola': MPA11, MPA17, MPA18, MPA26, MPA31, MPA34, MPA35, MPA36, MPA37, MPA38, MPA39, MPA40, MPA41, MPA42 and MPA43; these genotypes are kept at the experimental area of the 'Núcleo de Melhoramento Genético e Biotecnologia', Cruz das Almas, Bahia State, Brazil.

For the morphological characterization, the pollen grains were fixed in a modified Karnovsky (1965) solution [glutaraldehyde (2%), paraformaldehyde (2%), calcium chloride (0.001 M), sodium cacodylate buffer (0.05 M)] at pH 7.2 for 48 hours, dehydrated in an ascending ethanol series and dried in HMDS (hexamethyldisilazane). The samples were mounted in metal supports and coated with gold. The images were obtained with a variable pressure scanning electron microscope (LEO 435 VP, Carl Zeiss, Jena, Germany).

Pollen grains were subjected to weak lactic acetolysis to measure the pollen grains and exine (Raynal & Raynal, 1979). Twenty-five randomly selected pollen grains were used to measure the polar diameter, equatorial diameter and exine. The images were obtained with a photomicroscope (BX51, Olympus, Tokyo, Japan) coupled to a Sony camera using the software Image Pro-plus, v. 3.0. The terminology used to describe the pollen follows that of Punt, Hoen, Blackmore, Nilsson and Le Thomas (2007) and Hesse et al. (2009).

For the *in vitro* germination assays, pollen grains not subjected to any aseptic processes were inoculated in 35 mL of the different culture media: combination of two concentrations of sucrose (150 and 200 g L^{-1}), three concentrations of boric acid (0.1, 0.2 and 0.3 g L^{-1}), three concentrations of calcium nitrate (0.3, 0.4 and 0.5 g L^{-1}), three concentrations of magnesium sulfate (0.214, 0.314 and 0.414 g L^{-1}), three concentrations of potassium nitrate (0.1, 0.2 and 0.3 g L^{-1}) and a control without the use of the substances. All media were solidified with 0.8% agar, and the pH was adjusted to 7.0.

For each Petri dish, a sample consisting of pollen grains collected at anthesis from three flowers per raceme from thirty plants of each genotype was used. After the inoculation of the pollen grains, the Petri dishes were kept under controlled temperature conditions (27±1°C) in the dark for 24 hours. The germinated pollen grains were counted and the pollen tube length was measured using a binocular stereomicroscope.

To calculate the *in vitro* germination percentage, 100 randomly selected pollen grains from the Petri dishes were counted; the lengths of five randomly selected pollen tubes from each Petri dish were measured, for a total of 40 tubes per genotype. The pollen grain was considered germinated when its pollen tube diameter was equal to or larger than the pollen itself (Chagas et al., 2010).

The experimental design was completely randomized, with a 15 x 10 factorial arrangement (genotypes x culture media) with eight replicates (i.e., eight Petri dishes). The percentage data were arc-sine transformed ($\sqrt{x}\ 100^{-1}$) and subjected to

analysis of variance using the Scott-Knott test ($p \leq 0.01$). The analyses were performed using the SAS software (Statistical Analysis System [SAS], 2010).

The colorimetric analyses of pollen grains were performed using acetocarmine (2%) and 2,3,5-triphenyltetrazolium chloride (TTC) (1%) staining. One pollen sample collected from three flowers of each genotype was distributed over a glass slide; a drop of the specific dye was then placed on the slide, and a coverslip was added. In the case of the TTC dye, to allow the enzymatic reaction to occur, the amount of viable and non-viable pollen grains per genotype was determined two hours after the slides were prepared. For the acetocarmine dye, the analysis was performed shortly after staining.

To obtain a random sample of stained pollen grains, the slide-scanning method was used with an optical microscope; 100 pollen grains/slide/ genotype were counted with three replicates each, for a total of 300 pollen grains.

The experimental design was completely randomized in a 15x2 factorial scheme (genotypes x dyes) with three replicates each. Data were subjected to analysis of variance and means were compared by the Scott-Knott test ($p \leq 0.01$) using the SAS software (SAS, 2010).

Results and discussion

All genotypes were of a medium size, with an isopolar, subspheroidal (oblate spheroidal to prolate spheroidal) shape, radial symmetry, circular ambit, 3-colporate pollen grains with narrow colpi, absent margins, absent opercula, elongated endoapertures, tectate exines and granulated sexines (Figure 1). The thickness of the exine varied from 1.25±0.36 µm in genotype MPA35 to 2.35±0.75 µm in genotype MPA36 (Table 1). The similarity of the pollen of the different genotypes corroborates data from the literature, indicating that similar morphologies are expected in the same species (Cracraft, 2000).

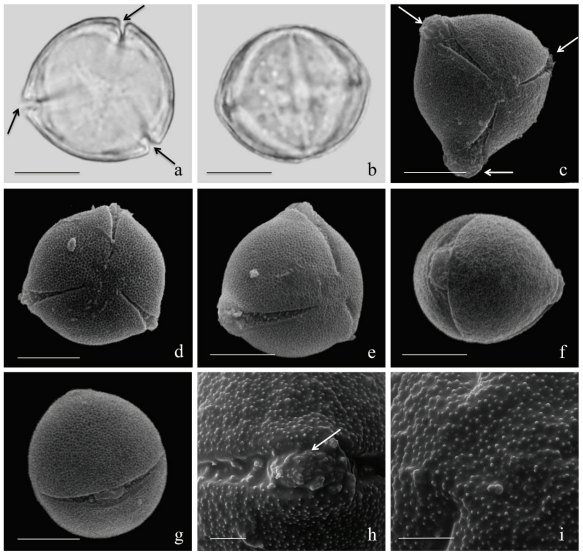

Figure 1. Morphology of pollen grains of the castor bean (*Ricinus communis* L.). a) Polar view of the acetolyzed pollen grain of genotype MPA38 by light microscopy (LM); the three colpi are indicated (arrows). b) Equatorial view of the acetolyzed pollen grain of genotype MPA40 by light microscopy (LM). c-g) Polar and equatorial views of pollen grains of genotypes MPA11 (c) MPA17 (d) MPA18 (e) MPA26 (f) and MPA31 (g) by scanning electron microscopy (SEM); the colpi are indicated (arrows). h) Detailed view of the colpus of genotype MPA31 by SEM (arrow). i) Detailed view of the exine ornamentation of genotype MPA18 by SEM. Bars: a-g = 10 µm, h-i = 3 µm.

Data obtained from the *in vitro* germination tests indicated that the genotype, culture media and genotype x culture media interaction had significant effects on the germination of pollen grains and the length of the pollen tube of the castor bean (Table 2).

Table 1. Morphology and morphometry of pollen grains of the castor bean (*Ricinus communis* L.) in equatorial view.

Genotypes	Polar diameter (PD)	Equatorial diameter (ED)	Exine	PD/ED[1]	Shape[2]
		µm			
MPA11	28.70±1.60	27.56±1.83	2.26±0.65	1.04	prolate spheroidal
MPA17	23.71±6.02	26.54±5.99	2.23±0.97	0.89	oblate spheroidal
MPA18	29.91±2.00	30.69±2.31	1.60±0.38	0.97	oblate spheroidal
MPA26	30.26±1.62	31.03±2.05	1.89±0.62	0.97	oblate spheroidal
MPA31	31.73±1.96	31.20±2.12	2.11±0.67	1.02	prolate spheroidal
MPA34	31.96±2.05	32.17±2.01	1.32±0.38	0.99	oblate spheroidal
MPA35	30.58±1.80	30.63±1.91	1.25±0.36	0.99	oblate spheroidal
MPA36	25.96±1.90	29.47±1.45	2.35±0.75	0.88	oblate spheroidal
MPA37	30.20±1.72	30.57±1.66	1.34±0.36	0.98	oblate spheroidal
MPA38	26.96±2.18	29.72±1.79	2.24±0.65	0.90	oblate spheroidal
MPA39	26.70±2.29	30.10±2.51	2.30±0.71	0.88	oblate spheroidal
MPA40	30.95±1.59	31.70±1.81	1.90±0.58	0.97	oblate spheroidal
MPA41	29.40±2.07	29.91±1.62	1.43±0.45	0.98	oblate spheroidal
MPA42	31.76±2.07	31.42±1.83	1.53±0.41	1.01	prolate spheroidal
MPA43	30.37 ± 1.86	33.26 ± 2.18	1.37 ± 0.35	0.91	oblate spheroidal

n = 25 replicates ± standard deviation. [1]Ratio of polar diameter to equatorial diameter in equatorial view. [2]According to nomenclature adopted by Punt et al. (2007) and Hesse et al. (2009).

Table 2. Percentage of *in vitro* germination and pollen tube length (mm) of genotypes of the castor bean (*Ricinus communis* L.) in different culture media.

GEN	Culture media									
	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10
					In vitro germination (%)					
MPA11	3.62aG	16.50aE	9.75aF	14.75aE	59.25eC	82.00aB	92.62aA	55.00cC	40.87dD	41.00cD
MPA17	0.00bE	3.75cD	2.62cD	2.12cD	29.87gB	15.87gC	18.37gC	31.87dB	43.12dA	51.50bA
MPA18	0.00bD	5.00cC	6.75bC	5.25bC	24.75gB	29.37fB	32.50fB	51.87cA	53.00cA	26.75dB
MPA26	0.00bH	7.50cG	5.25bG	5.87bG	48.87fD	44.75eD	38.37fE	79.12bA	68.25bB	57.87bC
MPA31	3.12aE	10.25bD	12.50aD	6.62bE	50.87fB	63.75cA	55.25dB	50.12cB	43.50dC	53.12bB
MPA34	0.00bF	0.00dF	0.00dF	0.00dF	44.75fD	70.00bC	82.00bB	92.37aA	62.37cB	77.00aB
MPA35	0.00bE	6.87cD	6.00bD	5.37bD	64.87dC	80.37aB	78.25bB	78.50bB	58.37cC	78.50aB
MPA36	3.75aE	18.62aD	16.87aD	17.75aD	86.25bA	73.00bB	78.62bB	91.12aA	79.37aB	49.25bC
MPA37	0.00bG	7.00cF	10.50aE	5.25bF	62.62dB	18.75gD	43.37eC	77.37bA	40.75dC	25.00dD
MPA38	0.00bG	7.62cF	9.00aF	4.87bF	51.37fC	38.00eD	38.50fD	84.12bA	71.00bB	55.12bC
MPA39	3.25aF	11.87bE	12.75aE	11.50aE	81.25bA	55.62dB	34.75fC	58.50cB	55.62cB	58.62bB
MPA40	0.00bG	11.37bE	9.62aE	4.00bF	74.62cB	63.62cC	56.00dC	80.62bA	70.50bB	80.87aA
MPA41	0.00bD	0.00dD	0.00dD	0.00dD	72.62cA	56.37dB	31.37fC	46.37cB	52.37cB	55.12bB
MPA42	5.37aG	12.37bF	5.62bG	8.37bG	90.37aA	80.75aB	69.50cC	27.00dE	59.12cD	79.50aB
MPA43	0.00bG	0.00dG	0.00dG	0.00dG	69.62cA	67.37cA	45.25eC	28.75dD	13.62eF	22.00dE
					CV(%) 15.17					
					Pollen tube length (mm)					
MPA11	0.068bE	0.121bC	0.114aD	0.109bD	0.192bA	0.155cB	0.143cB	0.130cC	0.132eC	0.125dC
MPA17	0.000cF	0.098cE	0.103bE	0.101bE	0.237aA	0.189bB	0.170bC	0.169aC	0.196aB	0.172aC
MPA18	0.000cE	0.100cD	0.094bD	0.095cD	0.165dB	0.182bA	0.163bB	0.156bB	0.174bA	0.185aA
MPA26	0.000cF	0.121bD	0.124aD	0.102bE	0.213bB	0.221aB	0.236aA	0.186aC	0.184bC	0.182aC
MPA31	0.076bD	0.117bB	0.093bC	0.092cC	0.146eA	0.137dA	0.118dB	0.146bA	0.122eB	0.128dB
MPA34	0.000cE	0.000dE	0.000cE	0.000dE	0.118fA	0.093fB	0.101eA	0.107eA	0.069hC	0.090fB
MPA35	0.000cF	0.125bD	0.094bE	0.121aD	0.198bA	0.159cB	0.104eE	0.137cC	0.114fD	0.154bB
MPA36	0.078bG	0.108cF	0.089bG	0.082cG	0.198bA	0.198bA	0.177bB	0.180aB	0.160cC	0.144cD
MPA37	0.000cF	0.132aD	0.101bE	0.087cE	0.196bA	0.168cB	0.148cC	0.179aB	0.176bB	0.158bC
MPA38	0.000cF	0.123bD	0.128aD	0.109bE	0.204bA	0.169cD	0.155cC	0.158bC	0.174bD	0.134dD
MPA39	0.067bE	0.100cD	0.096bD	0.117aC	0.181cA	0.153cB	0.115dC	0.116dC	0.108fC	0.108eC
MPA40	0.000cF	0.145aC	0.089bE	0.080cE	0.185cA	0.123eD	0.115dD	0.131cC	0.143dC	0.140cC
MPA41	0.000cD	0.000dD	0.000cD	0.000dD	0.168dA	0.145dB	0.171bA	0.144bB	0.163cA	0.121dC
MPA42	0.089aD	0.101cC	0.079bD	0.122aB	0.183cA	0.129eB	0.119dB	0.094fC	0.111fB	0.118dB
MPA43	0.000cD	0.000dD	0.000cD	0.000dD	0.106fA	0.086fB	0.069fC	0.086fB	0.090gB	0.090fB
					CV (%) 27.77					

Means followed by the same column lowercase letter and row uppercase letter belong to the same group by the Scott-Knott test ($p \leq 0.01$). M1 = Control; M2 = 0.1 g L^{-1} (H_3BO_3) + 0.3 g L^{-1} ($Ca(NO_3).4H_2O$) + 0.214 g L^{-1} ($Mg(SO_4).7H_2O$) + 0.1 g L^{-1} (KNO_3); M3 = 0.2 g L^{-1} (H_3BO_3) + 0.4 g L^{-1} ($Ca(NO_3).4H_2O$) + 0.314 g L^{-1} ($Mg(SO_4).7H_2O$) + 0.2 g L^{-1} (KNO_3); M4 = 0.3 g L^{-1} (H_3BO_3) + 0.5 g L^{-1} ($Ca(NO_3).4H_2O$) + 0.414 g L^{-1} ($Mg(SO_4).7H_2O$) + 0.3 g L^{-1} (KNO_3); M5 = 150 g L^{-1} sucrose + 0.1 g L^{-1} (H_3BO_3) + 0.3 g L^{-1} ($Ca(NO_3).4H_2O$) + 0.214 g L^{-1} ($Mg(SO_4).7H_2O$) + 0.1 g L^{-1} (KNO_3); M6 = 150 g L^{-1} sucrose + 0.2 g L^{-1} (H_3BO_3) + 0.4 g L^{-1} ($Ca(NO_3).4H_2O$) + 0.314 g L^{-1} ($Mg(SO_4).7H_2O$) + 0.2 g L^{-1} (KNO_3); M7 = 150 g L^{-1} sucrose + 0.3 g L^{-1} (H_3BO_3) + 0.5 g L^{-1} ($Ca(NO_3).4H_2O$) + 0.414 g L^{-1} ($Mg(SO_4).7H_2O$) + 0.3 g L^{-1} (KNO_3); M8 = 200 g L^{-1} sucrose + 0.1 g L^{-1} (H_3BO_3) + 0.3 g L^{-1} ($Ca(NO_3).4H_2O$) + 0.214 g L^{-1} ($Mg(SO_4).7H_2O$) + 0.1 g L^{-1} (KNO_3); M9 = 200 g L^{-1} sucrose + 0.2 g L^{-1} (H_3BO_3) + 0.4 g L^{-1} ($Ca(NO_3).4H_2O$) + 0.314 g L^{-1} ($Mg(SO_4).7H_2O$) + 0.2 g L^{-1} (KNO_3); M10 = 200 g L^{-1} sucrose + 0.3 g L^{-1} (H_3BO_3) + 0.5 g L^{-1} ($Ca(NO_3).4H_2O$) + 0.414 g L^{-1} ($Mg(SO_4).7H_2O$) + 0.3 g L^{-1} (KNO_3).

The pollen grain germination rates were highest in the M5 and M8 media, which differ only by the presence of sucrose. The M5 medium, which contains 150 g L^{-1} of sucrose, formed seven distinct groups. Five genotypes achieved germination rates above 70% (MPA36, MPA39, MPA41, MPA42 and MPA43) and the MPA42 genotype achieved a rate of 90.37% (Figure 2a). The M8 culture medium, which contains 200 g L^{-1} of sucrose, was defined by four groups and seven genotypes with *in vitro* germination rates above 70% (MPA26, MPA34, MPA35, MPA36, MPA37, MPA38 and MPA40); the MPA34 and MPA36 genotypes achieved particularly high germination rates of 92.37 and 91.12%, respectively. However, the germination rates of the MPA40 genotype did not differ from those obtained with the M10 culture medium.

The lowest rates of *in vitro* pollen grain germination were obtained with the M1 culture. Two groups formed with this medium: a group composed of the genotypes MPA11, MPA31, MPA36, MPA39 and MPA42, with germination rates ranging from 3.12 to 5.37%, and a group composed of the remaining genotypes that did not germinate *in vitro* (Table 2, Figure 2b). This result may be explained by the composition of the culture medium (water and agar only) and suggests that castor bean pollen requires other chemical elements to produce the pollen tube.

Different culture media have been used to germinate the pollen grains of a large number of species *in vitro* (Chagas et al., 2010; Cuchiara et al., 2012; Soares et al., 2008; 2013). Several authors have used carbohydrates and germination-stimulating substances (nutrients and hormones) in culture medium. Several organic and inorganic substances, such as sucrose, boric acid, calcium nitrate, potassium nitrate and magnesium sulfate, affect the *in vitro* germination of pollen grains (Galletta, 1983).

Sucrose, as a carbohydrate source, is added to culture media to either meet the metabolic needs of the explants by participating in the generation of energy and/or serve as a source of carbon skeletons

for biosynthetic processes involved in cellular differentiation (Chagas et al., 2010; Figueiredo, Pio, Silva, & Silva, 2013).

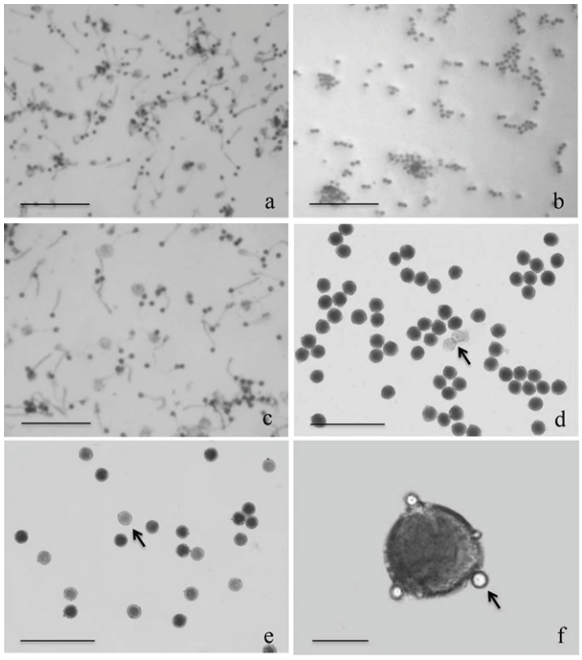

Figure 2. *In vitro* germination (a-c) and colorimetric analysis (d-f) of pollen grains of the castor bean (*Ricinus communis* L.). a) Genotype MPA42 in M5 culture medium showing high percentages of germination. b) Genotype MPA17 in M1 culture medium, with the absence of germination. c) Genotype MPA17 in M5 culture medium showing longer pollen tube lengths. d) Viable and non-viable (arrow) pollen grains stained with acetocarmine. e) Viable and non-viable (arrow) pollen grains following staining with TTC. f) Presence of pollenkitt (arrow) in pollen grain stained with acetocarmine. Bars: a-c = 0.5 mm; d-e = 200 μm; f = 20 μm.

Greater pollen tube growth was observed in culture medium M5 (Figure 2c) for all of the genotypes except genotype MPA26, which showed a longer pollen tube (0.236 mm) in medium M7, and genotype MPA18 in media M6 (0.182 mm), M9 (0.174 mm) and M10 (0.185 mm), which belong to the same group (Table 2).

The smallest pollen tubes were observed in the M1 medium, and most of the genotypes produced no germinated pollen in this medium. Germination occurred for genotypes MPA34, MPA41 and MPA43 only in medium M5, indicating that the absence of sucrose completely inhibited germination. According to Scorza and Sherman (1995), good pollen must have 50 to 80% germinated pollen grains with well-developed pollen tubes. Most of the genotypes analyzed in this study could be used as male parents for genetic improvement or conservation programs in germplasm banks; the only exception was genotype MPA17, which achieved low pollen grain germination rates (from 0 to 51% *in vitro*) (Table 2). This pattern can be explained by several factors, including genetic origin, environmental conditions and an inappropriate germination medium.

In vitro germination provides a controlled experimental system; however, this system does not completely reproduce *in vivo* pollen tube growth, as interactions may occur between ingredients of the culture medium and different plant materials. Nevertheless, according to Soares et al. (2008), *in vitro* germination produces results that are relatively close to those that would be expected *in vivo*. For this reason, it is important to develop a well-adjusted methodology for each studied species.

The analysis of viability with acetocarmine identified three groups (Table 3). The first group consisted of eleven genotypes (MPA11, MPA17, MPA31, MPA34, MPA36, MPA37, MPA38, MPA39, MPA41, MPA42 and MPA43) with viabilities greater than 96%. The second group consisted of genotypes MPA26, MPA35 and MPA40 with viabilities of 91.67, 95 and 93.67%, respectively. The third and last group consisted of only one genotype (MPA18) with a viability of 64.66%. It is important to note that the acetocarmine dye is a marker of the integrity of chromatin and stains pollen grains deep red; in the presence of this dye, non-viable grains remain transparent (Figure 2d). The results found in this study corroborate those obtained by Vargas, Souza, Silva and Bobrowski (2009) who analyzed pollen viability in four cultivars of castor bean using acetocarmine and observed viabilities of more than 86.46%.

Table 3. Viability of pollen grains of the castor bean (*Ricinus communis* L.) genotypes by colorimetric analysis.

Genotypes	Viability (%)	
	Acetocarmine	TTC
MPA11	98.67aA	95.67bB
MPA17	98.00aA	60.00eB
MPA18	64.67cA	58.33eA
MPA26	91.67bB	99.00aA
MPA31	96.67aA	97.67bA
MPA34	97.67aA	77.67dB
MPA35	95.00bB	99.33aA
MPA36	97.00aA	87.00cB
MPA37	96.33aA	93.00cA
MPA38	97.00aA	73.00dB
MPA39	97.00aA	88.00cB
MPA40	93.67bA	95.00bA
MPA41	98.33aA	71.67dB
MPA42	98.00aA	98.33aA
MPA43	99.33aA	87.00cB
CV %	3.09	

Means followed by the same column lowercase letter and row uppercase letter belong to the same group by the Scott-Knott test ($p \leq 0.01$).

The pollen viability of genotype MPA17 (98%) was overestimated by the acetocarmine assay, as the TTC assay yielded a viability (60%) closer to that

observed in the *in vitro* germination (51.50%) in culture medium M10. Viability results for genotype MPA18 obtained with the dye assays and the *in vitro* culture system with media M8 (51.87%) and M9 (53%) were similar.

The TTC assay identified five groups of genotypes, with pollen germination rates varying from 58.33 to 99% (Table 3). Pollen grains stained light red were considered viable, whereas non-viable pollen showed grayish tones (Figure 2e).

The viability of a given genotype differed with different dyes, and the acetocarmine appeared to overestimate the viability (over 90% for most genotypes). Similar results were observed by Munhoz, Luz, Meissner Filho, Barth and Reinert (2008), who found that acetocarmine staining overestimated the viability of papaya pollen relative to TTC.

In this study, it was evident that the viability assay using TTC was more reliable, as the results obtained with this dye were similar to those obtained in the *in vitro* germination system. This dye is a marker for the activity of dehydrogenase enzymes involved in the respiratory activity of living tissues. The enzymatic activity present in the pollen grain is associated with the germination capacity of the pollen. Several authors have argued that the TTC test is a reliable estimate of pollen viability and provides results close to those obtained in *in vitro* germination tests (Huang, Zhu, Mu, & Lin, 2004; Munhoz et al., 2008).

The *in vitro* germination rates and the results obtained in the colorimetric analysis are directly related (Scorza & Sherman, 1995). However, it has been suggested that the dye method overestimates and the *in vitro* germination method underestimates the percentage of germinated pollen (Galletta, 1983). Einhardt, Correa and Raseira (2006) compared the methods used to test the viability of peach pollen and concluded that the use of the *in vitro* germination method provides satisfactory results relative to the *in vivo* germination method and that propionic carmine staining overestimates the percentage of viable pollen grains.

Exudates were observed in the exine (external surface) of the pollen grain of the castor bean; these exudates were most likely a lipophilic substance known as pollenkitt (Figure 2f), which is very common in members of the Euphorbiaceae family (Vargas et al., 2009). Pollenkitt protects and minimizes the dehydration of pollen grains and consequently limits the loss of viability in this species; additionally, this compound is involved in promoting the adhesion of grains to the stigma, inducing the volatilization of compounds and attracting pollinators due to its color (Souza, Pereira, Viana, Silva, & Sudré, 2004).

Conclusion

Similar pollen morphology was observed in the fifteen studied genotypes.

The M5 and M8 media were the most efficient media used in the *in vitro* germination system.

Acetocarmine and 2, 3, 5-triphenyltetrazolium chloride (TTC) staining showed that the castor bean pollen grains of the studied genotypes had high levels of and great variation in pollen viability at anthesis.

All of the studied genotypes have high levels of pollen viability and can therefore be used as male parents in genetic improvement programs.

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