academicJournals

Vol. 14(14), pp. 1207-1214, 8 April, 2015
DOI: 10.5897/AJB2015.14449
Article Number: C04048A52179
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Effect of collection time on the viability of banana pollen grains

Taliane Leila Soares[1*], Everton Hilo de Souza[1]*, Laecio Fernandes Souza Sampaio[2], Maria Angélica Pereira de Carvalho Costa[2], Sebastião de Oliveira e Silva[2] and Janay Almeida dos Santos-Serejo[1]

[1]Embrapa Cassava and Fruits, Brazilian Agricultural Research Corporation, Cruz das Almas, BA, 44380-000, Brazil.
[2]Center of Agricultural, Environmental and Biological Sciences, Federal University of Recôncavo da Bahia, Cruz das Almas, BA, 44380-000, Brazil.

Received 27 January, 2015; Accepted 30 March, 2015

The objective of this study was to assess the pollen viability of six improved diploid banana plants (AA) collected in different periods of the day, in two seasons of the year (winter and summer), using *in vitro* germination test and staining. Pollen grains collected at 8 am (anthesis), 10 am, 12 pm, 2 pm and 4 pm were evaluated. We used a culture medium for pollen germination containing 15% sucrose, 0.01% H_3BO_3, 0.01% KNO_3, 0.03% $Ca(NO_3)_2.4H_2O$, and 0.02% $MgSO_4.7H_2O$, solidified with 0.8% agar, adjusted to different pH. The pollen viability was evaluated by staining with 2,3,5-triphenyltetrazolium chloride (TTC). The highest pollen germination rates and viability were obtained at 8 am and the lowest at 4 pm, in both seasons. The average *in vitro* germination percentage and viability level were negatively influenced by the number of hours after anthesis. During the summer, the pollen viability and *in vitro* germination rates were highest when compared to winter period. The results presented can help at the selection of genetic materials and enable inferences on the best pollen collection time for use in cross breeding programs for plant improvement.

Key words: *Musa* spp., *in vitro* pollen germination, 2,3,5-triphenyltetrazolium chloride.

INTRODUCTION

In Brazil, the conventional banana breeding program is achieved by successive crossings, seeking to obtain tetraploid hybrids by crossing diploid and triploid cultivars. For this purpose, special attention is focused on the diploid germplasm (AA), since it concentrates the largest number of desirable traits, such as parthenocarpy, good number of hands, long fingers (fruits), well-formed bunches, low plant height and resistance to pests and

*Corresponding author. E-mail: talialeila@gmail.com. Tel: +55 75 92562577.

Abbreviations: TTC, 2,3,5-Triphenyltetrazolium chloride; **RH,** relative humidity; **H_3BO_3,** boric acid; **KNO_3,** potassium nitrate; **$Ca(NO_3)2.4H_2O$,** calcium nitrate tetrahydrate; **$MgSO_4.7H_2O$,** magnesium sulfate heptahydrate; **HCl,** hydrochloric acid.

diseases (Amorim et al., 2013). Therefore, it is essential to know the pollen fertility of diploid hybrids, since they will be used as male genitors in genetic improvement programs. Analysis of the viability of pollen supplies an indication of the male fertility, allowing better targeting and more security in the crosses carried out, increasing the efficiency of obtaining desirable hybrids. The viability of pollen can be determined using direct methods, such as inducing germination *in vitro* (Jiang et al., 2009; Alcaraz et al., 2011; Rodriguez-Enriquez et al., 2013) and *in vivo* (Dane and Ekici, 2011; Fakhim et al., 2011), or by indirect methods based on cytological parameters, such as staining (Burke et al., 2007; Abdelgadir et al., 2012; Bhat et al., 2012; Ćalić et al., 2013). Pollen vigor is a prerequisite for the fertilization and development of seeds. In the research of tissue culture, pollen germination rate and the length of pollen tube were usually used as indicators of pollen vigor (Ma et al., 2012). Therefore, studies of pollen growth in culture media, the determination of the best collection times and the histochemical analysis of pollen are fundamental in reproductive biology and genetic improvement to obtain the highest pollen viability and develop new hybrids with traits of agronomic interest. Additionally, other factors that can influence pollen germination are the nutritional state of the plant, season of the year, time of day, temperature and pH of the culture medium (Boavida and McCormick, 2007; Soares et al., 2008; Daher et al., 2009; Distefano et al., 2012; Rodriguez-Enriquez et al., 2013).

The pH of germination medium is an important factor controlling pollen germination and pollen tube development in different plant species (Qiu et al., 2005; Acar et al., 2010; Boavida and McCormick, 2007; Zaman et al., 2009; Conner, 2011; Rodriguez-Enriquez et al., 2013). Also, it has influence on the nutrient availability, plant regulators amount and the degree of agar solidification. Several studies have showed data on the pH ranges and optimum values for pollen germination and pollen tube in different species (Munzuroglu et al., 2003; Qiu et al., 2005; Zaman, 2009). However, for banana, little information exists about the effect of pH on *in vitro* pollen germination (Soares et al., 2008). In any pollination method, pollen viability is considered satisfactory when between 50 and 70% of the pollen germinate. In the field, the pollen of some species only become viable several hours after release and remain so only for short time, during which the temperature must be moderate, the humidity high and the light intensity sufficient for germination to occur. As the pollen grains get older, the percentage of germination and the average of tube length decline. Even when pollen appears otherwise unviable, the presence of some vigorous tubes indicates that they can produce effective fructification, albeit with low germination percentage (Scorza and Sherman, 1995). Knowledge of the best time to collect pollen to obtain high viability is important to produce hybrid seeds, particularly of species where artificial hybridization is possible. Although, studies have been published on methods to assess the viability of banana pollen, to date there are no reports to our knowledge of the influence of the time of pollen collection along the day and the year on hybridization efficiency.

Therefore, this study aimed to determine the best moment to collect pollen from diploid banana plant genotypes with high viability using *in vitro* germination and staining with 2,3,5-triphenyltetrazolium chloride (TTC). Pollen was collected at 5 time points (8 am, 10 am, 12 pm, 2 pm and 4 pm) during two seasons of the year (Winter and Summer).

MATERIALS AND METHODS

Materials

The study was carried out in the experimental field of the Embrapa Cassava and Fruits, located at 12° 40' S. latitude and 39° 06' W. longitude, in Cruz das Almas, Bahia, Brazil. The climate in the region, according to the Köppen classification, is a transition between Am and Aw zones, with average annual rainfall of 1,143 mm, average temperature of 24.28°C and relative humidity (RH) of 60.47%. The experiments were conducted separately in the winter (June, 22.1°C, 87, 6% RH) and summer (December, 25.3°C, 76,2% RH).

Plant material

We used pollen from six improved banana plant diploid genotypes (AA), generated by the banana improvement program of Embrapa Cassava and Fruits (Table 1). The pollen grains were collected from flowers in anthesis, removed from the same bract, at five different times of the day (8 am, 10 am, 12 pm, 2 pm and 4 pm). Then pollen grains collected at each time point were placed *in vitro* in a culture medium for germination and tube growth evaluation.

Methods

In vitro pollen germination and pollen tube length evaluation

For *in vitro* germination assays, pollen grains that were not subjected to any aseptic processes were inoculated in Petri dishes (9 cm diameter and 1 cm height) containing 35 mL of the culture medium proposed by Soares et al. (2008). Briefly, the medium is composed of 15% sucrose, 0.01% boric acid, 0.01% potassium nitrate, 0.03% calcium nitrate and 0.02% magnesium sulfate, solidified with 0.8% agar and adjusted at pH of 5.8 or 7.0 before autoclaving at 121°C for 20 min. For each Petri dish we used a pool composed of pollen from five flowers of each genotype. After inoculation, the pollen grains were kept at 27±1°C in the dark for 24 h. The germinated pollen grains were then counted, and the pollen tube length was measured using a binocular stereomicroscope. The experimental design was completely randomized using a 6 × 5 × 2 factorial arrangement (genotype × collection time × pH), with eight replicates. To calculate the *in vitro* germination percentage, 100 randomly selected pollen grains were counted. Regarding pollen tube length, five randomly selected pollen tubes from each replicate were measured (representing 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 (Tuinstra and Wedel, 2000).

Table 1. Banana diploid genotypes (AA) used in this study and their respective parents.

Improved diploid genotype	Female parent	Male parent
013018-01	Malaccensis (W)	Sinwobogi (C)
042052-04	M 53 (H)	Kumburgh (C)
050012-02	M 61 (H)	Lidi (C)
088079-01	Malaccensis (W) x Madang (C)	Tuu Gia (C) x Calcutta 4 (W)
089087-01	Malaccensis (W) x Sinwobogi (C)	Calcutta 4 (W) x Heva (C)
091087-01	Borneo (W) x Guyod (C)	Calcutta 4 (W) x Heva (C)

W = Wild; C = Cultivar; H = Hybrid, with unknown parents.

Histochemical analysis

The histochemical analysis of the pollen was performed by staining them with 2,3,5-triphenyltetrazolium chloride (TTC) diluted to 1% in Tris buffer (HCl 0.15 M, pH 7.8). The TTC is an enzymatic test used to detect the dehydrogenase enzyme activity of cells. Pollen grains removed from three anthers of flowers collected from the same bract were distributed on glass slides and stained with a drop of the stain solution and covered with a slip. Observations of the number of viable and unviable pollen grains for each genotype were carried out 2 h after preparation of the slides because TTC requires a time interval for enzymatic reaction to occur. To obtain a good representativeness of the viability of the pollen grains, 100 pollen grains/slide/genotype were counted (three replicates each, for a total of 300 pollen grains) using an optical microscope. Results are expressed in percentage. Pollen grains stained by TTC in light red or dark red were counted as viable, while noncoloured were classified as unviable (Duro et al., 2013). The experimental design was completely randomized in a 6 × 5 factorial scheme (genotype × collection time) with three replicates each.

Data analysis

Percentages obtained were transformed to arc sin ($\sqrt{x/100}$) before the statistical analysis. To assess the relationship of *in vitro* germination and the histochemical test results, we applied analysis of variance, while we used the Tukey test to compare the means at 5% probability. To complement the statistical analysis we used regression analysis to choose mathematical models with the best fit, according to the highest values of the coefficient of determination (R^2) and the F-test, both at 5% significance. We used the SAS System, version 9.2 (SAS Institute, 2010) for all statistical analyses.

RESULTS

In vitro pollen germination and pollen tube length evaluation

The data obtained for the *in vitro* germination indicated a significant effect ($p<0.001$) of all the factors studied taken independently (genotype, collection time and pH), as well as a significant effect for the interaction between those factors and the two seasons (winter and summer) (Tables 2 and 3). Regarding the *in vitro* germination assay, percentage was higher in the summer than in the winter (Table 1). For all genotypes, the germination rate was in general highest for the pollen collected at 8 am and placed in the culture medium with pH 7.0 whatever the season. Among the banana diploids studied, genotype 089087-01 presented the highest germination rates, greater than 90%, when the flowers were collected at 8 am and pollen grains were placed in culture medium with pH 7.0, for both winter and summer season (Figure 1a to 1b). On the other hand, genotype 050012-02 presented the lowest germination percentages at all collection times, both in summer and winter (Figure 1c). Pollen collected in summer grew longer tubes than those collected in the winter (Table 3). Diploid 042052-04 presented the longest tube lengths, with averages of 4.80 mm in summer and 3.42 mm in winter (Figure 1d). Pollen tube growth was always longer for pollen collected at 8 am with pH 7.0 medium, except for the genotype 050012-2 where pollen tube was longer in pH 5.8 medium.

Histochemical analysis

Data obtained by histochemical staining with TTC are in agreement with data obtained on *in vitro* germination test. Indeed, genotype 089087-01 stood out, with 91.33 and 86.00% viable pollen from flowers collected at 8 am in the Summer and Winter, respectively (Table 4, Figure 1e). On the other hand, diploid 050012-02 presented the lowest viability values at all collection times, in both seasons (Figure 1f). Regardless the season for all genotypes, pollen grains collected at 8 am exhibited the highest viability values. The regression analysis showed that in both seasons, the germination rate, tube length and pollen viability, as determined by histochemistry, were negatively influenced by the time lapse from anthesis (Figures 2 and 3). There was a linear relation for germination rate, tube length and histochemistry result (y) and collection times (x), with the highest averages obtained at 8 am and the lowest at 4 pm. This behavior can be explained by the fact that the pollen grains collected at 8 am were at their peak of physiological development (anthesis), after which these values declined during the day.

DISCUSSION

In vitro germination in culture medium is a technique that simulates the conditions of the style-stigma, inducing

Table 2. Percentage of *in vitro* pollen germination from diploid banana plants (AA), collected at different times of the day in two different seasons (winter and summer).

Collection time	Genotypes											
	013018-01		042052-04		050012-02		088079-01		089087-01		091087-01	
	pH 5.8	pH 7.0	pH 5.8	pH 7.0	pH 5.8	pH 7.0	pH 5.8	pH 7.0	pH 5.8	pH 7.0	pH 5.8	pH 7.0
Winter												
8 am	46.36^{aB}	69.41^{aA}	55.42^{aB}	78.79^{aA}	14.99^{aB}	23.94^{aA}	31.22^{aB}	48.50^{aA}	55.47^{aB}	90.75^{aA}	49.44^{aB}	80.05^{aA}
10 am	37.19^{bB}	47.84^{bA}	40.25^{bB}	53.11^{bA}	11.21^{aB}	18.77^{aA}	20.75^{bB}	29.45^{bA}	37.42^{bB}	62.53^{bA}	32.77^{bB}	44.58^{bA}
12 am	29.55^{cB}	39.13^{cA}	33.40^{cB}	44.51^{cA}	6.56^{bB}	12.21^{bA}	15.53^{bB}	21.98^{cA}	33.02^{bB}	49.34^{cA}	26.91^{cB}	35.99^{cA}
2 pm	22.41^{dB}	28.84^{dA}	27.62^{dA}	32.76^{dA}	3.66^{bA}	7.49^{cA}	10.74^{cA}	15.39^{dA}	28.07^{cB}	40.07^{dA}	25.59^{cB}	35.11^{cA}
4 pm	15.96^{eA}	18.99^{eA}	18.77^{eA}	22.29^{eA}	2.95^{bA}	5.95^{cA}	10.42^{cA}	11.64^{dA}	17.34^{dB}	25.06^{eA}	15.35^{dA}	20.35^{dA}
CV (%)	10.62											
Summer												
8 am	53.75^{aB}	76.50^{aA}	64.37^{aB}	84.50^{aA}	32.87^{aB}	41.50^{aA}	39.12^{aB}	52.37^{aA}	66.00^{aB}	96.37^{aA}	51.50^{aB}	76.37^{aA}
10 am	44.00^{bB}	52.62^{bA}	51.87^{bB}	66.37^{bA}	29.00^{aB}	40.12^{aA}	29.75^{bB}	43.25^{bA}	51.37^{bB}	77.00^{bA}	40.87^{bB}	59.50^{bA}
12 am	34.25^{cB}	44.87^{cA}	43.50^{cB}	52.37^{cA}	21.37^{bB}	29.62^{bA}	28.62^{bB}	38.50^{bA}	44.25^{cB}	57.87^{cA}	31.50^{cB}	51.50^{cA}
2 pm	31.00^{cB}	36.25^{dA}	31.87^{dB}	41.75^{dA}	10.50^{cB}	23.50^{cA}	24.12^{bB}	30.00^{cA}	37.00^{dB}	51.87^{dA}	25.00^{dB}	41.00^{dA}
4 pm	21.87^{dB}	29.50^{eA}	24.87^{eB}	35.00^{eA}	5.50^{cB}	19.75^{cA}	8.87^{cB}	25.75^{cA}	29.75^{eB}	39.50^{eA}	21.87^{dB}	35.12^{eA}
CV (%)	10.26											

Means followed by the same lower-case letters in the column and upper-case letters in the row within the same factor do not differ from each other by the Tukey test at 5% probability. Pollen germination was assessed in culture media described by Soares et al. (2008). Two different pH were tested (pH: 5.8 and 7.0).

Table 3. Measurement of pollen tube length (mm) from diploid banana plants (AA) collected at different times of the day in two different seasons (winter and summer).

Collection time	Genotype											
	013018-01		042052-04		050012-02		088079-01		089087-01		091087-01	
	pH 5.8	pH 7.0	pH 5.8	pH 7.0	pH 5.8	pH 7.0	pH 5.8	pH 7.0	pH 5.8	pH 7.0	pH 5.8	pH 7.0
Winter												
8 am	2.17^{aB}	2.87^{aA}	2.68^{aB}	3.42^{aA}	1.01^{bA}	0.91^{aA}	0.91^{aA}	1.08^{aA}	1.16^{aA}	1.32^{aA}	1.03^{abB}	1.80^{abA}
10 am	1.90^{aB}	2.71^{aA}	2.14^{bB}	2.71^{bA}	1.05^{bA}	0.81^{aB}	0.81^{aA}	0.77^{bA}	0.77^{bA}	0.92^{bA}	1.09^{aB}	2.03^{aA}
12 am	1.35^{bB}	2.08^{bA}	1.62^{cB}	2.24^{cA}	1.37^{aA}	0.80^{aB}	0.80^{aA}	0.70^{bA}	0.85^{bA}	0.77^{bcA}	0.80^{bcB}	1.72^{bA}
2 pm	1.04^{cB}	1.43^{cA}	1.07^{dA}	1.14^{dA}	0.90^{bcA}	0.67^{aB}	0.75^{aA}	0.64^{bA}	0.68^{bA}	0.58^{cA}	0.74^{bcB}	1.08^{cA}
4 pm	0.75^{cB}	1.37^{cA}	0.96^{dB}	1.40^{dA}	0.69^{cA}	0.75^{aA}	0.67^{aA}	0.65^{bA}	0.61^{bA}	0.55^{cA}	0.70^{cB}	1.04^{cA}
CV (%)	38.82											
Summer												
8 am	2.50^{aB}	3.83^{aA}	3.36^{aB}	4.80^{aA}	1.66^{aA}	1.19^{aB}	1.19^{aB}	1.61^{aA}	1.29^{aB}	1.76^{aA}	1.29^{aB}	2.86^{aA}
10 am	2.56^{aB}	3.19^{bA}	2.56^{bB}	3.62^{bA}	1.63^{aA}	1.14^{aB}	1.14^{aA}	1.10^{bA}	1.14^{aA}	1.33^{bA}	1.30^{aB}	2.67^{aA}
12 am	1.94^{bB}	2.56^{cA}	2.15^{cB}	2.70^{cA}	1.66^{aA}	1.20^{aB}	1.20^{aA}	1.04^{bA}	0.80^{bB}	1.26^{bA}	1.06^{abB}	2.25^{bA}
2 pm	1.30^{dB}	2.10^{dA}	1.55^{dB}	2.37^{cA}	1.27^{bA}	0.95^{aB}	0.95^{aA}	0.88^{bA}	0.57^{bB}	0.85^{cA}	0.92^{bB}	1.51^{cA}
4 pm	1.03^{dB}	1.95^{dA}	1.50^{dB}	2.00^{dA}	0.87^{dA}	0.88^{aA}	0.88^{aA}	0.80^{bA}	0.51^{bB}	0.83^{cA}	0.90^{bB}	1.40^{cA}
CV (%)	32.10											

Means followed by the same lower-case letters in the column and upper-case letters in the row within the same factor do not differ from each other by the Tukey test at 5% probability. Pollen germination was assessed in culture media described by Soares et al. (2008). Two different pH were tested (pH: 5.8 and 7.0).

germination and pollen tube growth. Each species requires a specific protocol of culture medium to obtain adequate pollen grain germination (Soares et al., 2013). The pH of the culture medium considered ideal for *in vitro*

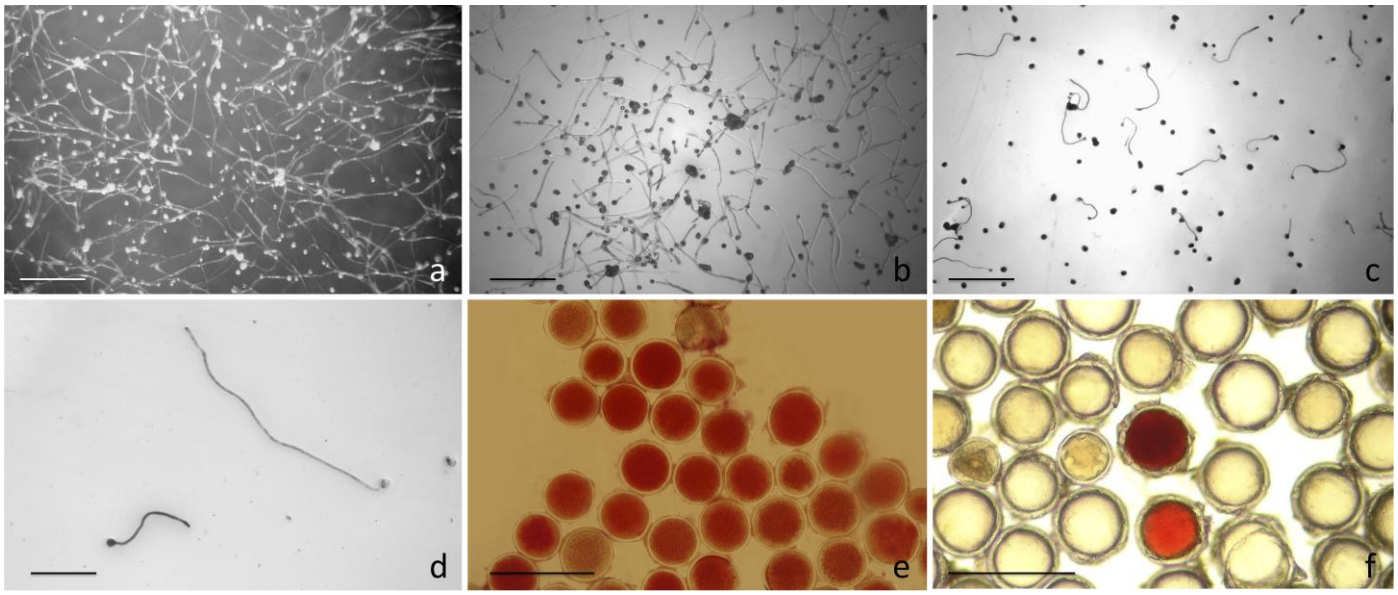

Figure 1. Viability of the pollen from diploid banana plants. Germination of pollen from genotype 089087-01 in culture medium with pH 7.0, from flowers collected at 8 am in summer (a) and winter (b). Germination of pollen from genotype 050012-02 in culture medium with pH 5.8, from flowers collected at 8 am in winter (c). Greater length of the pollen tube of genotype 042052-04 in culture medium with pH 7.0, from flowers collected at 8 am in summer (d). Histochemical test with TTC on genotype 089087-01, evidencing the high viability of the pollen when collected at 8 am in summer (e). Histochemical test with TTC on genotype 050012-02, evidencing the low viability of the pollen when collected at 8 am in winter (f). Bars: a-d = 2 mm; e-f = 200 µm.

Table 4. Percentage of pollen viability from diploid banana plants (AA) determined by the histochemical test with TTC (2,3,5-triphenyltetrazolium chloride) at 1% both in Winter and Summer.

Genotype	8 am	10 am	12 am	2 pm	4 pm
Winter					
013018-01	57.83^{dA}	55.20^{cA}	42.73^{cB}	37.30^{cB}	38.27^{bB}
042052-04	75.00^{bA}	63.13^{bB}	56.10^{bB}	46.43^{bC}	42.73^{aC}
050012-02	34.13^{fA}	24.43^{dB}	17.90^{dC}	17.80^{eC}	11.70^{dD}
088079-01	46.60^{eB}	51.43^{cA}	41.43^{cB}	33.03^{cD}	34.70^{cD}
089087-01	86.00^{aA}	73.43^{aB}	63.00^{aC}	51.30^{aD}	46.03^{aD}
091087-01	65.90^{cA}	55.80^{cB}	48.70^{cB}	41.73^{bC}	41.90^{aC}
CV(%)			4.26		
Summer					
013018-01	77.33^{bA}	67.00^{bB}	60.33^{bB}	53.00^{aC}	41.67^{aC}
042052-04	82.33^{bA}	75.00^{bA}	59.67^{bB}	49.00^{aC}	44.00^{aC}
050012-02	45.00^{dA}	35.00^{dB}	27.33^{bC}	24.67^{cC}	19.00^{cC}
088079-01	60.66^{cA}	52.33^{cB}	46.00^{cB}	36.00^{bC}	32.67^{bC}
089087-01	91.33^{aA}	84.00^{aA}	70.67^{aB}	58.00^{aC}	48.67^{aD}
091087-01	79.67^{bA}	68.00^{bB}	55.33^{bC}	41.00^{bD}	39.00^{bD}
CV (%)			7.25		

Means followed by the same lower-case letters in the column and upper-case letters in the row within the same factor do not differ from each other by the Tukey test at 5% probability.

pollen germination and pollen tube growth of different species of plants is situated near neutrality or in alkaline condition (Acar et al., 2010, Conner, 2011; Boavida and McCormick, 2007; Rodriguez-Enriquez et al., 2013). In banana, Soares et al. (2008) observed that the culture medium with pH 7.0 promoted greater pollen germination percentage. In this study, we showed that pH 5.8 of the culture medium negatively affected the *in vitro* pollen

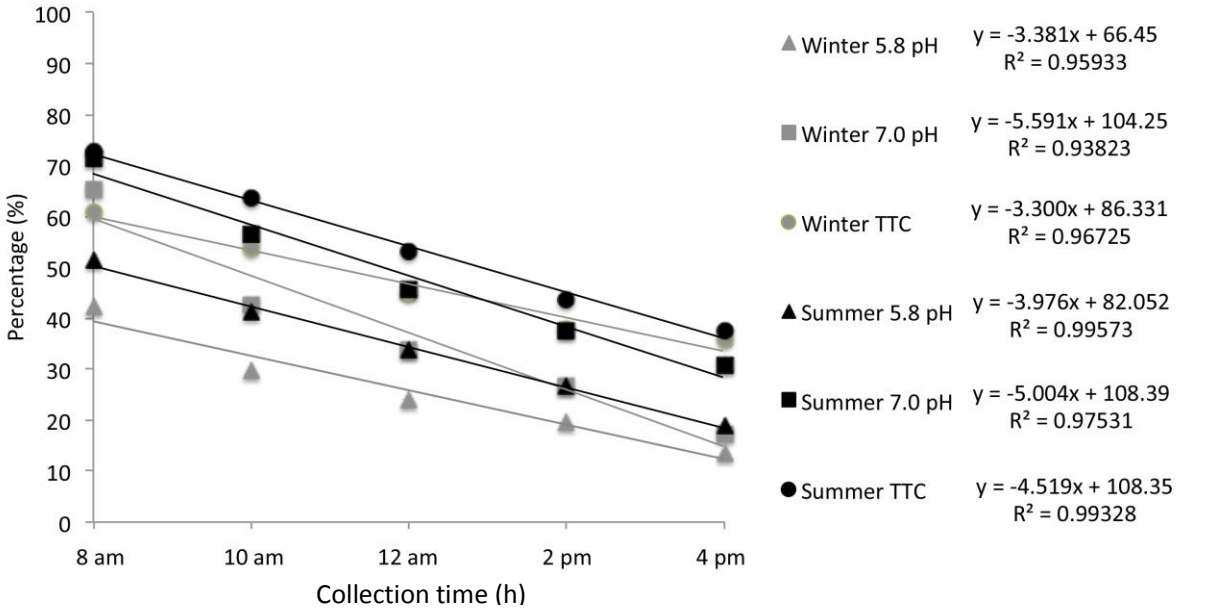

Figure 2. Percentage of pollen germination and pollen viability estimated by histochemistry, from diploid banana plants, calculated at 5 different time points during a day and for two seasons of the year (winter and summer). For pollen germination, tests were performed *in vitro* on culture media adjusted at pH: 5.8 and 7.0 as described by Soares et al. (2008).

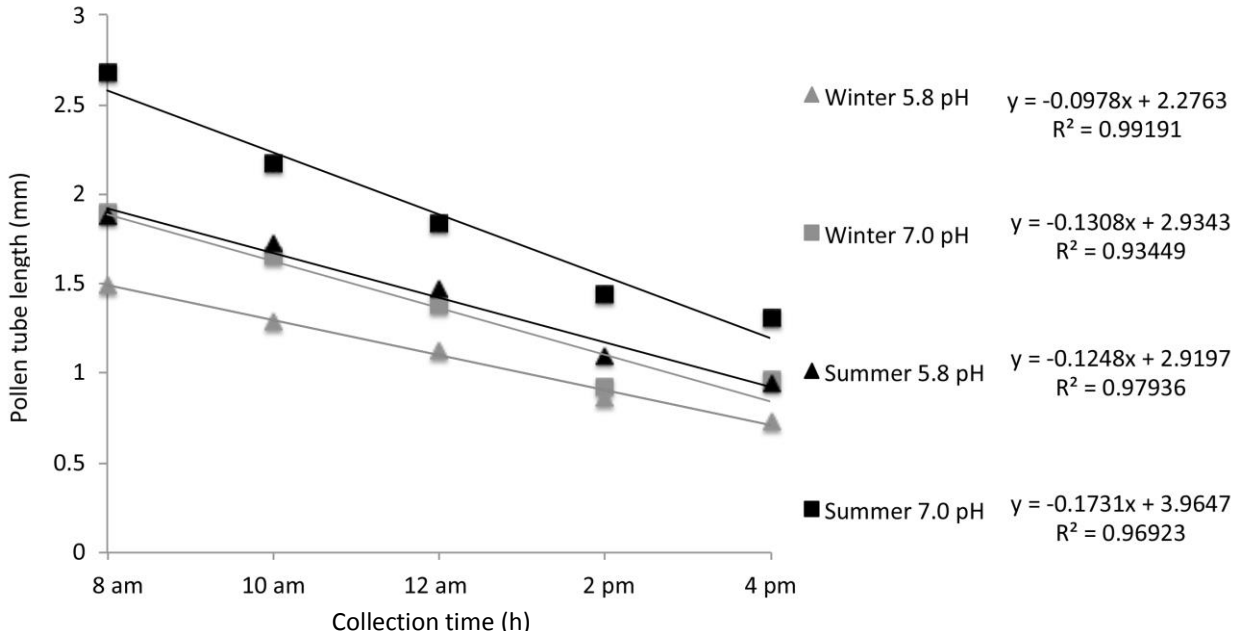

Figure 3. Pollen tube length (mm) of diploid banana plants expressed at five different time points during a day and for two seasons of the year (winter and summer). For pollen tube length measurements, tests were performed *in vitro* on culture media adjusted at pH: 5.8 and 7.0 as described by Soares et al. (2008).

germination and pollen tube growth when compared to pH 7.0, except for 050012-02 genotype which had highest pollen tube length in pH 5.8. Probably these differences in pH medium preferences for pollen tube growth are due to the great influence of the genetic variation among genotypes. Similarly, Sharafi et al. (2011) observed that pollen germination and pollen tube growth varied according to species, cultivar and method

that may have been used. Furthermore, Kakani et al. (2005) concluded that the differences observed in *in vitro* pollen germination and pollen tube growth of 12 cotton cultivars (*Gossypium hirsutum* L.) were reflections of the variability of the cultivars. In *Arabidopsis thaliana* (L.) Heynh., Costa et al. (2013) observed differences in the percentage of *in vitro* pollen germination and pollen tube growth in relation to the culture media. Other factors influenced *in vitro* pollen germination as incubation time after placing the pollen on the plates, development state of the flowers when collected and storage conditions (Qiu et al., 2005; Rodriguez-Enriquez et al., 2012). The maturation of pollen is one of the development stages of the life cycle of plants. *In vitro* germination does not occur within the anther, but the pollen must be ready to germinate soon after the anther's dehiscence (Lin and Dickinson, 1984). Therefore, hybridization will be more effective when using pollen collected at the optimal time of the day.

Veiga et al. (2012), studying corn pollen collected at different time points (9 am, 2 pm and 4 pm), found that the maize pollen collected in the morning presented higher germination rates than those collected in the afternoon. The results presented here show that the time of pollen collection in banana along the day affects its viability. We indeed, observed a maximum of pollen viability (Figure 2) when pollen was collected at 8 am whatever the genotypes tested. Loss of pollen viability in function of time after flower opening was also observed by Souza et al. (2002) in yellow passion fruit plants using histochemical analyses by Alexander (1980) and Lugol (Johansen, 1940), although the index remained high, above 75%, even 24 h after anthesis.

Judd et al. (1999) also established that the viability of pollen can be altered by variations in humidity and temperature, and this can be tested by means of germination capacity, enzyme activity and presence of a cytoplasm. According to the authors, this variation can be species-specific: for example, while the pollen of some grasses is only viable for a few minutes or hours, the pollen of other species can remain viable for many years if stored properly. Dusi et al. (2010) also found that the external environmental conditions influence the *in vitro* germination of pollen of *Brachiaria* spp. accessions. According to them, the pollen grains gathered during the rainy season or the day after a rainstorm are unable to germinate. According to Zonia et al. (2002), pollen tubes tend to rupture because of the increase of hydrostatic pressure and low cell wall resistance. This allows a rapid inflow of water in the pollen, causing the loss of soluble substances and ions in the cytoplasm, known as "imbibition damage" (Loguercio, 2002). Some of our preliminary studies (data not presented) go in the same direction because we observed inefficient germination and intense eclosion after rainy days. For these reasons, we did not collect pollen on rainy days. *In vitro* germination rates and viability according to histochemical analysis are directly related (Scorza and Sherman, 1995). In general, the results obtained in the present study are in agreement with that statement, given that our pollen germination data indicated a significantly lower rate in pH 5.8 than that observed when using staining materials. Other authors also have reported that the observations from histochemical tests lead to overestimation of the pollen germination percentage, while *in vitro* test results cause underestimation (Sutyemez, 2011; Coser et al., 2012).

Although, the culture medium used in the *in vitro* pollen germination may simulate the pistil conditions, it is impossible for *in vitro* method to display all conditions that affect pollen tube growth *in vivo* (Hedhly et al., 2005). The assessment of viability using TTC is based on the color change of the tissues in the presence of a salt solution of 2,3,5-triphenyltetrazolium chloride, which is reduced by dehydrogenase respiratory enzymes in live tissues, resulting in a red carmine-colored compound called formazan (Beyhan and Serdan, 2008). The TTC test has been used to estimate the pollen viability in many species (Kelen and Demirtas, 2003; Huang et al., 2004; Kang et al., 2009; Soares et al., 2013). Several authors argued that the TTC test is a reliable estimative of pollen viability, showing similar results to *in vitro* germination tests (Bolat and Pirlak, 1999; Huang et al., 2004). In addition, the TTC is widely used because it is relatively fast and easy method. Abdelgadir et al. (2012) observed that only TCC staining was able to differentiate viable and unviable pollen in *Jatropha curcas* L. (Euphorbiaceae). Similarly, Huang et al. (2004), who studied the viability of *Leymus chinensis* (Trin.) Tzvelev pollen using the TTC stain was able to distinguish the relative decline of the pollen viability percentage after anthesis. Knowledge of the adequate moment to collect pollen is essential for genetic improvement programs involving banana plants, mainly to obtain more efficient crosses. In the present study, we observed that the best time to collect pollen for hybridization is at 8 am on the day of floral anthesis and that *in vitro* germination and growth of the pollen tube are much better in summer time. The pollen collected at that time showed high viability in the test with TTC staining and the highest *in vitro* germination percentage, both in the winter and summer.

Conflict of interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors are grateful to the Brazilian Federal Agency for the Support and Evaluation of Graduate Education (CAPES) by granting a doctoral fellowship for T.L. Soares.

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