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FLORAL PHASE AND SUCROSE/BORIC ACID CONCENTRATIONS ON *in vitro* GERMINATION OF *Inga edulis* (Mart.), POLLEN GRAIN

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ABSTRACT - The aim of this work was to determine the ideal stage of pollen collection and the best concentrations of sucrose and boric acid in the culture medium for the *in vitro* germination of inga fruit tree pollen. For this, the work was carried out in two stages, one using concentrations of sucrose in the culture medium (0, 5, 10, 20 and 30%) - experiment 1. Another one using the best sucrose concentration, with concentrations (0, 100, 200 and 300 mg L^{-1}) - experiment 2. In the two experiments, two flowering phases were use: pre-anthesis and post-anthesis. For both experiments, the culture medium contained 1% agar to solidify the medium and all treatments remained in BOD for 24 h at a temperature controlled at 25°C. For the experiment 1 with sucrose, DIC was use in factorial 2 x 5, with 4 replicates, and for the experiment 2 with boric acid, DIC was use in factorial 2 x 4, with 4 replicates. Data were submit to analysis of variance and regression. For pollen grains obtained from pre-anthesis, the maximum technical efficiency point (PMET) was obtain with 11% sucrose added to the culture medium, thus achieving 33% germination. As for pollen grains obtained from flowers in post-anthesis, PMET was reach with 8% of sucrose in the culture medium, obtaining 32% of germination. Within the concentrations evaluated in the experiment, boric acid did not promote increase of *in vitro* germination of pollen from inga fruit tree.
Keywords: Native fruit tree, Culture medium, Pollen tube.

FASE FLORAL E CONCENTRAÇÕES DE SACAROSE/ÁCIDO BÓRICO NA GERMINAÇÃO in vitro DE GRÃOS DE PÓLEN DE Inga edulis (Mart.),

RESUMO - O trabalho objetivou determinar o estágio ideal de coleta do pólen e as melhores concentrações de sacarose e ácido bórico no meio de cultura para a germinação *in vitro* do pólen do ingá fruit tree. Para isso, o trabalho foi realizado em duas etapas, uma utilizando concentrações de sacarose no meio de cultura (0, 5, 10, 20 e 30%) - experimento 1 e outra utilizando a melhor concentração de sacarose combinada com concentrações de ácido bórico (0, 100, 200 e 300 mg L^{-1}) - experimento 2. Nos dois experimentos foram utilizadas duas fases de floração: pré-antese e pós-antese. Para ambos os experimentos o meio de cultura continha 1% de ágar, para solidificar o meio e todos os tratamentos permaneceram em BOD por 24 h, com temperatura controlada a 25°C. Para o experimento 1 com sacarose foi utilizado DIC, em fatorial 2 x 5, com 4 repetições, e para o experimento 2 com ácido bórico, foi utilizado DIC em fatorial 2 x 4, com 4 repetições. Os dados foram submetidos à análise de variância e de regressão. Para grãos de pólen obtidos de flores em pré-antese, o ponto de máxima eficiência técnica (PMET) é obtido com 11% de sacarose adicionado ao meio de cultura, alcançando assim 33% de germinação. Para grãos de pólen obtidos de flores em pós-antese, o PMET é alcançado com 8% de sacarose no meio de cultura, obtendo-se 32% de germinação Dentro das concentrações avaliadas no experimento, o ácido bórico não promoveu aumento da germinação de pólen de ingá fruit tree.
Palavras-chave: fruteira nativa, meio de cultura, tubo polínico.

INTRODUCTION

Present in the tropical forests of South and Central America (POMPEU et al., 2012), the species *Inga edulis* Mart. has a wide use in the Brazilian Amazon region, due to its versatility of use, such as high calorific coal and fast-growing wood, potential for biomass production used in green fertilization, high development in the restoration of degraded soils, as well as, in the participation of agroforestry systems (LOJKA et al., 2010). It also observes the consumption of its fruits, because they have sweet white, velvety pulp, suitable for human consumption or when already passed are given to animals to supplement the diet, as an energy and proteins source.

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The *I. edulis* propagation method employed is by seed (SOUZA et al., 2010). However, the genus *Inga* has characteristic of presenting recalcitrant seeds, which gives them sensitivity to desiccation, as well as, if stored at low temperatures can lead loss of viability, which makes them difficult to maintain in the long term (FONSECA; FREIRE, 2003).

In order for seed production to be satisfactory, pollination must be carefully observed (NASCIMENTO et al., 2012). In addition to seed production normally in the plant, pollination followed by fertilization allows the production of hybrids in breeding programs (EINHARDT et al., 2006). However, it considers the need for prior knowledge of the using material, thus requiring feasibility tests. Therefore, for Costa et al. (2009), testing viability ways can be given in four ways, through tests with specific dyes, *in vitro* germination, *in vivo* germination and percentage of effective fruiting.

In vitro germination, it is attempt to artificially reproduce stigma and stylet conditions, inducing the germination of the pollen tube (ALMEIDA et al., 2011). In addition, the necessary components for germination in a culture medium where the pollen remains for a certain time period for growth the pollen tube (FONSECA et al., 2010).

In this sense, in vitro germination is a process that involves the germination of pollen sample in appropriate culture medium, but that will vary from species to species (SALLES et al., 2006). However, it notes that for pollen germination of all angiosperms a source of energy is required, which will usually have some variations of sugars (sucrose, glucose), boron or boric acid and other nutrients such as calcium. Sucrose, in addition to providing energy, facilitates the osmotic balance between the pollen grain and the culture medium, and together with boron, react more rapidly with the cell membranes, facilitating the growth of the pollen tube (FIGUEIREDO et al., 2013).

Therefore, the importance of estimating pollen viability is to allow the gene flow analysis in plants, through the expression of the male reproductive species potential, helping in breeding programs, taxonomic, ecological, genetic studies, among others (FRESCURA et al., 2012). In addition, it helps to select superior genotypes in breeding programs, through controlled pollination, since pollen viability has a direct influence on successful in vitro fertilization (HISTER; TEDESCO, 2016).

For *I. edulis* there are not still pollen viability studies testing different culture media. Therefore, the present work aimed to estimate the pollen viability of *I. edulis* by testing different culture media with different concentrations of sucrose and boron for different phases of flowering of the plant (pre-anthesis and post-anthesis).

MATERIAL AND METHODS

The experiment was carried out at the Laboratory of Plant Physiology of the Universidade Tecnológica Federal do Parana (UTFPR), *Campus* Dois Vizinhos. Pollen from two matrices of I. edulis that had present a balloon stage, both located at the university headquarters. The study was in two steps, one using different sucrose concentrations in the medium (experiment 1) and the other using the optimal concentration of sucrose (already selected in experiment 1) with different concentrations of boric acid (experiment 2). To the two experiments were used two phases of flowering: pre-anthesis and post-anthesis.

In experiment 1, different concentrations of sucrose (0, 5, 10, 20 and 30%) were test for two phases of flowering, pre-anthesis and post-anthesis. For this retreated plant branches in the field, containing flowers stint flask, which were transport to the laboratory and placed for drying paper trays on silica chamber at room temperature (25°C) until there was natural opening of the anther until it reaches maturity and release of the pollen grain.

For the pre anthesis stage, the pollen was extract before the flask opened, with the aid of sieve and for the post-anthesis stage the pollen was collect four hours after the anthesis. After extraction with the aid of brushes number two sterilized by the mane pony the pollen spread on slides, which were contain in the number two sterilized by the mane pony the pollen spread on slides, which were contain in the different culture medium was test according to the concentrations of sucrose and 1% agar. The same was do for the post-anthesis stage, right after the opening of the flowers. Then, the slides were put in the boxes of type Gerbox® (polystyrene boxes, square, 11 cm and 3.5 cm high, containing lids), where it contained water film on moist paper to maintain humidity. Soon after, then these were bring to BOD (Biological Demand Oxygen), where it remained for 24 hours, with temperature, at 25°C.

The same methodology was used in experiment two, using different concentrations of boric acid (0, 100, 200, and 300 mg L^{-1}) and the best sugar concentration of the previous sucrose experiment, in the pre- and post-anthesis phases.

After 24 h, the pollen grains were counted from each slide, with the aid of optical microscope with 400x magnification (10 x 40 x eyepiece of the lens), counting polyads 50 grouping of pollen grains (BARROS et al., 2013). In each polyade, there was a differentiated number of pollen grains. In this way, the average number of pollen grains per polyade was determined, reaching the result of 25 pollen grains by pollen and between germinated and non-germinated pollen grains. In experiment 1, was use a completely randomized delimitation with 2 x 5 factorial (stage of flower development x sucrose concentration), with four replications, each represented by Petri® plate. In experiment 2, the experimental design wascompletely randomized delimitation, in factorial 2 x 4 (flower development stage x concentrations of boron), with four replicates, each represented by de Petri® plate. Pollen grains in which the length of the pollen tube was equal to or greater than the diameter of the pollen grain was considered as germinated (FRANZON; RASEIRA, 2006).

The data were submitted to the normality test (Lilliefors) and homogeneity of variance (Bartllet). Given the assumptions of the model, they were submitting to analysis of variance (ANOVA) to verify the significance

of the factors and their interactions. When significant, it was applied regression analysis to the quantitative factors, using aid Gene's software (CRUZ, 2013).

There was a significant interaction at the 1% probability level of the error between floral development stage (A) and sucrose concentration (B), rejecting the hypothesis of nullity *H0* (Table 1).

RESULTS AND DISCUSSION

TABLE 1 - Anova: coefficient of variation (CV), degrees of freedom (DF) and average squares (AS) of the analysis of variance for the variable pollen germination, in an experiment conducted in DIC with four replicates.

Sources of variation	DF	AS
Stage of floral development (A)	1	168.1000^{ns}
Concentration of sucrose (B)	4	1815.4125*
A x B	4	662.6625*
Block	3	77.2333^{ns}
Residue	27	43.5481
CV (%)	35,77	

*Significant at the 5% probability level of error by the F test (0,01 = < p <0,05). ns = not significant (p > = 0,05).

The equations regression was significant at the 5% probability level by the T test. For pollen grains obtained from pre-anthesis flowers, the Maximum Technical Efficiency Point (PMET) was obtain with 11% sucrose added to the culture medium, thus achieving 33% germination. For pollen grains obtained from post-anthesis flowers, PMET was reach with 8% sucrose in the culture medium, obtaining 32% of germination (Figure 1).

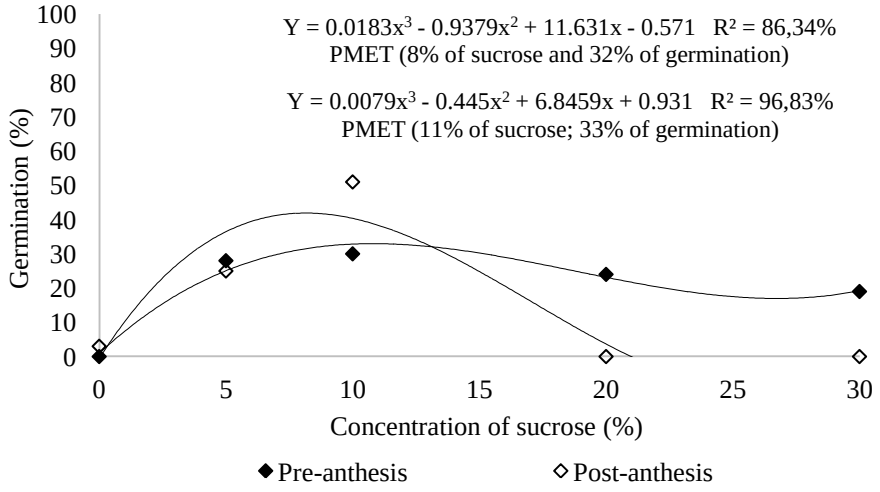

FIGURE 1 - *In vitro* germination of *I. edulis* pollen according to the concentrations of sucrose in the culture medium.

It could be observed that the highest germination days were obtain with the presence of sucrose, as explained by Chagas et al. (2010) result that an ideal concentration of sucrose increases the availability of carbohydrate, providing more energy for cellular biosynthesis processes. They found in their study positive effects on germination of pollen grains of 'Taiwan Nashi-C' pear tree rootstocks when sucrose concentrations of up to 47.78 g L^{-1} were add.

Salles et al. (2006) found similar value of sucrose concentration in the culture medium working with different concentrations of sucrose in the germination of citrus pollen grains, where it states that the best sucrose concentration for the species studied is 100 gL^{-1}. Concentration similar to that found in the present work for in vitro germination of *I. edulis* pollen in the pre-anthesis phase.

Derin and Eti (2001) observed higher in vitro germination rates for pomegranate pollen grains in culture medium containing a concentration of 10 g L^{-1} sucrose. Dantas et al. (2005) studying the in vitro germination of pollen on apple tree different sucrose concentrations they conclude that the germination of pollen grains in apple cultivars tested by the authors, sucrose at concentrations between 15% and 25% has the highest germination rates.

Value close to that found in this work of 8% sucrose concentration in the culture medium was observed by Sinimbú Neto et al. (2011), working with in vitro germination of pollen grains of bacuri tree, where they affirm that there is a positive relation between germination percentage and sucrose concentration in a medium containing up to 7.5 % of sucrose.

The behavior observed on the stage of floral development may be variable among species, as well as in

Eugenia involucrate (cherry fruit forest tree), in which Franzon and Raseira (2006) verified better germination percentages in pollen grains from pre- anthesis flowers.

As the germinative percentage obtained was below the value considered satisfactory (80%) (SCORZA; SHERMAN, 1995), the effect of adding boric acid to the culture medium was evaluated, taking into account the best results obtained previously.

The addition of boric acid to the culture medium did not prove to be efficient on the germination of grains of *I. edulis* pollen under the conditions evaluated. PMET was achieve in this case when 15 mg.L^{-1} is used of boric acid added to the culture medium, reaching only 14% of germination (Figure 2).

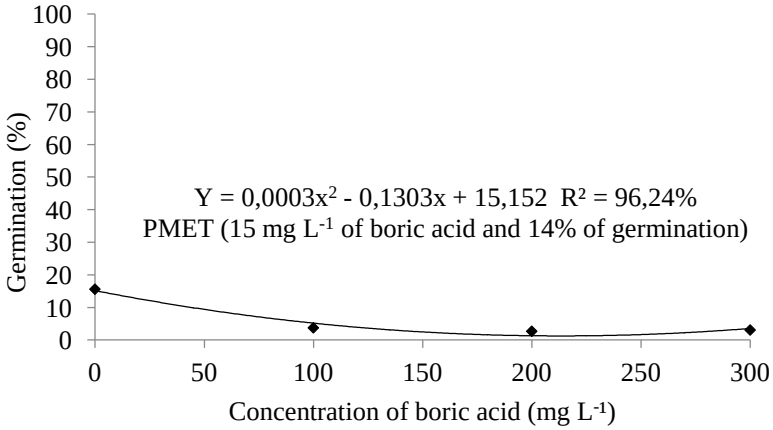

FIGURE 2 - *In vitro* germination of *I. edulis* pollen according to the concentrations of boric acid in the culture medium.

Dantas et al. (2005) when tested the viability of pollen in apple tree, with addition of boric acid did not obtain positive effects. When germination was evaluated in the absence of boric acid and in the presence of 15% sucrose, the highest percentages of germination were found Fuji (51.1%), Imperatriz (31.7%), M-9 (20.8% %), Catarina (19.2%), Gala (13.7%) and Marubakaido (6.1%). Ramos et al. (2008), testing the germination of citrus pollen grains with different concentrations of boron, affirm that the necessity of the addition of this nutrient can vary between different species and varieties.

The decrease in germination of the inga fruit tree when boric acid is added might be related to what was described by Luz et al. (2008), where they report that the absence, as well as the excess of boric acid can act negatively on the osmotic equilibrium between the pollen grain and the culture medium and lead to a rupture of the pollen tube.

It was recommend for future studies the evaluation of other factors that might contribute to the pollen germination process, such as the use of other sources of sugar and other nutrients available in the culture medium, pollen grains dehydration, incubation temperature in the moment of germination, among others.

CONCLUSIONS

It was recommending the use of 11% sucrose for *in vitro* germination of *I. edulis* pollen, regardless of the stage of floral development.

Within the evaluated concentrations, the use of boric acid did not promote improvements on the germination percentage of pollen.

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