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In vitro viability of pollen grain of fiji dwarf coconut accession

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ABSTRACT

Lethal yellowing is a disease that causes severe damages to coconut palms and it has devastated the coconut canopy of several Caribbean islands, and areas of southern Florida. The Fiji dwarf coconut accession has demonstrated tolerance to this disease. Pollen storage and germination provides useful information for conservation strategy and genetic improvement programs. The objective of this study was to evaluate pollen viability of Fiji dwarf coconut by *in vitro* germination under different nutritional and abiotic conditions. For the first experiment, two liquid culture media were evaluated for *in vitro* germination of Fiji dwarf coconut pollen; the Lora culture medium and the Armendariz culture medium combined with four temperature levels. In the second experiment, different sucrose concentrations in the Lora culture medium (4, 8, 12 and 16%) were evaluated combined with two incubation times (6 and 24 h) in the presence or absence of light. The highest *in vitro* germination of pollen grain was obtained in Lora culture medium by 24 h incubation period at 28 ºC. The light condition does not influence the germination process. *In vitro* germination percentage improved as the sucrose concentration increased according to a linear regression. In contrast, damage pollen grain percentage reduced according to a quadratic regression.

Index terms: *Cocos nucifera* L.; *in vitro* germination; inflorescence; conservation.

INTRODUCTION

The coconut palm (*Cocos nucifera* L.) belongs to the Arecaceae and palm trees of this family can be found in all tropical and subtropical areas of the world. The coconut palm is an attractive ornamental tree, but it is also widely used for food, in confectionery and beverages (Ree; Guerra, 2015), being also used as precursor of biodiesel (Nguyen et al., 2016). Coconut annual production is about 62,5 million fruits covering an area of about 11,80 million hectares, according to the Asian and Pacific Coconut Community (FAO, 2019).

Lethal yellowing disease is a destructive disease of coconut and poses a huge threat to the coconut industry wherever it occurs (Aidoo et al., 2021). The Fiji dwarf coconut (also known as Niu Leka) emerged as the prized jewel among varieties because of its heavy, dense crown of short, dark leaves—features especially sought by ornamental growers, landscapers and gardeners (Flores, 2008). Although it has variable LY resistance in Florida, it is free of nutritional deficiencies that plague most other coconut varieties grown on Florida's relatively infertile soils is a promising cultivar that shows tolerance to lethal yellowing and has ornamental use in South Florida (Flores, 2008). Small Fiji dwarf germplasm collections have been maintained in Florida at both the Tropical Research and Education Center, University of Florida, in Homestead, and the ARS-USDA Subtropical Horticultural Research Station.

Pollen conservation is an important tool in management of plant genetic resources (Dinato et al., 2020). Besides this, the efficacy of the crosses, both between varieties and cultivars of

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the same species and between different species, depends directly on the pollen grains viability (Soares et al., 2016). The development of reliable methods to determine the functional quality of pollen helps monitoring pollen viability during storage, and include studies of pollen interaction with stigma, optimization of culture media, as well as incompatibility and fertility studies (Shivanna; Rangaswamy, 1992). Pollen viability is associated with stigma receptivity, which, in turn, indicates the best period of pollen deposition in the flower (Brito et al., 2010). In additional, its viability can be evaluated using dyes, *in vitro* germination; *in vivo* germination, and percentage of effective fruit formation obtained through pollination. *In vitro* germination is efficient and offers rapid results (Einhardt; Correa; Raseira, 2006).

Some authors have suggested that the culture medium for *in vitro* germination should contain, besides the carbohydrates, germination-stimulating substances, such as boric acid, calcium nitrate, potassium nitrate and magnesium sulfate (Khan; Perveen, 2006).

Previous studies indicate variation of *in vitro* pollen germination responses as a function of the culture medium and the coconut genotype (Armendariz et al., 2006; Karun et al., 2006; Karun; Sajin, 2010; Machado et al., 2014; Moura; Machado; Ledo, 2015). Currently, there is no information available for *in vitro* pollen germination of Fiji dwarf coconut.

The sucrose concentration is an important factor due to promote osmotic balance between pollen and culture media and acts as a source of energy (Sarkar; Sarkar; Vangaru, 2018). Abiotic factors related to incubation conditions, as temperature and presence or absence of light can be considered for the pollen studies.

Therefore, the aim of this study was to optimize *in vitro* pollen germination for Fiji dwarf coconut accession under different nutritional and abiotic conditions.

MATERIAL AND METHODS

Plant material

Closed inflorescences (spathe) of Fiji dwarf coconut accession in mature stage from 3 to 4 months were manually collected from the germplasm collection at the Tropical Research and Education Center, University of Florida, in Homestead, Florida, USA. The spathes were maintained in a controlled environment growth chamber at 30 ºC for 72 h. After that, were removed from the growth chamber, opened and the pollen grains were collected from the male flowers and stored in 2-ml cryotubes. Subsequently were maintained in a refrigerator at 4 °C by 48 h following storage procedures developed by Machado et al. (2014).

Culture media and temperature levels

Two liquid culture media were evaluated for *in vitro* germination of Fiji dwarf coconut pollen. The Lora culture medium (Lora et al., 2006) and the Armendariz culture medium (Armendariz et al., 2006) were selected due to the previous results published for coconut varieties (Armendariz et al., 2006; Machado et al., 2014; Moura; Machado; Ledo, 2015). Both media compositions are described in Table 1. The pH was adjusted to 5.8 before autoclaving at 120 ± 1 °C for 20 min.

Table 1: Composition of Lora and Armendariz culture media.

Composition	Lora Culture Medium (Lora et al., 2006)	Armendariz Culture Medium (Armendariz et al., 2006)
$MgSO_4.7H_2O$ (mg L^{-1})	200	40
$Ca(NO_3)O_2.4H_2O$ (mg L^{-1})	300	86
KNO_3 (mg L^{-1})	100	20
H_3BO_3 (mg L^{-1})	100	100
Sucrose (%)	4	15

The *in vitro* pollen germination of Fiji dwarf coconut was evaluated using 1 mL of pollen solution (0.03 g of pollen in 10 mL of distilled water) placed onto Petri dishes containing 10 mL of culture medium. In addition to the culture media, different incubation temperature levels were evaluated: very low: 22 °C, low: 25 °C, room temperature: 28 °C, and high: 30 °C in a growth chamber under light intensity of 45.14 µmol m^{-2} s^{-1}, supplied by white fluorescent lamps. After 6 h incubation period, drops (100 µL) of culture medium and pollen were collected and deposited in three glass slides (26 x 76 mm). Pollen *in vitro* germination were analyzed in an optical microscope (Leica Microsystems®, Germany) with daylight blue microscope filter at

10X optical magnification. The germinated, non-germinated and damaged pollen were counted in three fields containing 100 pollen grains each and for the image capture was used SPOT software version 4.7. Pollen was considered germinated when the length of the pollen tube is larger than its diameter and damage when presents burst. To calculate the *in vitro* pollen germination, non-germinated and damage percentages the following formula were used:

$$in\ vitro\ \text{pollen germination}\,(\%) = \frac{\text{number of germinated pollen grains}}{\text{number of counted pollen grains}} x100$$

$$in\ vitro\ \text{pollen non}-\text{germinated}\,(\%) = \frac{\text{number of non}-\text{germinated pollen grains}}{\text{number of counted pollen grains}} x100$$

$$\text{damage pollen}\,(\%) = \frac{\text{number of damage pollen grains}}{\text{number of counted pollen grains}} x100$$

Sucrose concentration, light condition and incubation period for *in vitro* germination

Different sucrose concentrations in the Lora culture medium (4, 8, 12 and 16%) were evaluated. For pollen germination, 0.03 g of pollen were placed in 10 mL of distilled water and 1 mL was transferred into 2-mL Eppendorf tubes containing 1.0 mL of culture medium. The samples were maintained in an incubator (28 °C) in the presence and absence of light (45.14 µmol s^{-1} m^{-2}). After 6 and 24 h incubation periods, slides were prepared as previously described, with three repetitions per sample. Next, were analyzed under a microscope (Leica Microsystems, Germany) with daylight blue microscope filter at the 10X optical magnification, and the visual fields were evaluated and captured using the SPOT software version 4.7. The percentage of *in vitro* pollen grains germinated, non-germinated and damaged were evaluated.

Experimental design and statistical analysis

For the first experiment, the statistical design was completely randomized in a 2 x 4 factorial scheme (two culture media x four incubation temperature levels) with three repetitions, each composed of a Petri dish containing 100 grains of pollen counted in each field of the slide. The second, was completely randomized in a 4 x 2 x 2 factorial scheme (four sucrose concentrations x two light conditions x two incubation periods) with four repetitions, each composed of a Petri dish containing 100 grains of pollen in each field of the slide.

Data were analyzed by analysis of variance (ANOVA) and Tukey's Test was used to compare means ($p \leq 0.05$), for sucrose concentration was applied regression analysis. The data were performed using the Computer Programming System for Analysis of Variance - SISVAR (Ferreira, 2014).

RESULTS AND DISCUSSION

Effects of culture media and incubation temperature on the *in vitro* pollen germination

There was a significant difference for the pollen germination percentage and for the interaction between culture medium and incubation temperature factors (Table 2).

Table 2: Percentage of *in vitro* pollen germination of Fiji green dwarf coconut on Lora and Armendariz culture medium submitted at different incubation temperature levels.

Incubation Temperature Levels	Armendariz Culture Medium (Armendariz et al., 2006)	Lora Culture Medium (Lora et al., 2006)
Very Low - 22 °C	34.14 Aa	19.01 Bb
Low - 25 °C	33.30 Aa	29.81 Ba
Room Temperature - 28 °C	22.00 Bb	47.75 Aa
High - 31 °C	34.46 Aa	13.41 Bb
VC (%)	30.53	

Means followed by the same upper-case letter in rows and lower-case letter in columns are not significantly different from each other by Tukey's test at 5% probability.

The highest pollen germination percentage (47.75%) in Lora culture medium at room temperature (28 ºC) also was reported by Machado et al. (2014) in CRD (Cameroon red dwarf) coconut, where a pollen germination percentage of 49.64% (Figure 1). Furthermore, the Lora culture medium has been shown to be suitable for evaluating of *in vitro* pollen germination of the BGD (Brazilian Green Dwarf of Jiqui) and BT (Brazilian Tall) coconuts (Moura; Machado; Ledo, 2015). However, at the other incubation temperature levels (22, 25 and 31 °C) the Armendariz culture

medium showed higher *in vitro* pollen germination percentages ranged from 33.3 to 34.46% than the Lora culture medium. In contrast, Sousa et al. (2016) no observed positive correlation between viability and germination for different culture media on the pollen germination of oil palm (*Elaeis guineensis* Jacq).

Associated with mineral composition and concentration of culture medium is the range of temperature, which can also affect pollen germination. Probably the temperature for *in vitro* pollen germination of Fiji dwarf coconut may differ according to the plant's origin site microclimate. Higher levels of date palm (*Phoenix dactylifera* L.) pollen germination were measured at the 23 °C incubation conditions (52%) compared to 30 °C (39%) (Oliveira et al., 2021). For oil palm, the highest pollen *in vitro* germination (30.19%) was reached at 30 °C (Lin et al., 2017).

Establishing the different stages of pollen viability is also essential to achieve the best germination results. In general, each species has its own requirements regarding the composition of culture medium due to genetic variations between species and the osmotic pressure of pollen (Lin et al., 2017). The Lora culture medium (Lora et al., 2006) presents excellent nutritional conditions for the germination of pollen grains of the Fiji green dwarf coconut at room temperature (28 ºC), as demonstrated in the Figure 1.

Effects of sucrose concentration, light condition and incubation time on the *in vitro* pollen germination

There were significant differences in the responses (*in vitro* pollen germination, non-germinated and damaged) for incubation time and sucrose concentration, as isolated factor. For the light condition and double or triple interactions between the factors were not significant for all variables. The highest percentage of pollen *in vitro* germination (42.9%) occurred at 28 °C for 24 h incubation period (Table 3).

However, a different result was reported by Armendariz et al. (2006), for Malaysian green dwarf coconut (MGD). The pollen grains maintained for 6 h in the dark presented 40.1% of *in vitro* germination. According to Lin et al. (2017) the extrinsic factors can affect pollen germination are culture time, incubation temperature and culture medium.

The quality of pollen, often referred to as pollen fertility, is the result of a combination of different factors, such as viability of mature pollen and germination capacity through the formation and growth of *in vitro* conditions of the pollen tube. Pollen quality can be influenced by genetic, environmental (temperature and humidity) and agronomic factors (Lin et al., 2017; Pereira et al., 2018), and therefore incubation temperature plays a major role in improving *in vitro* germination percentages. Previous research

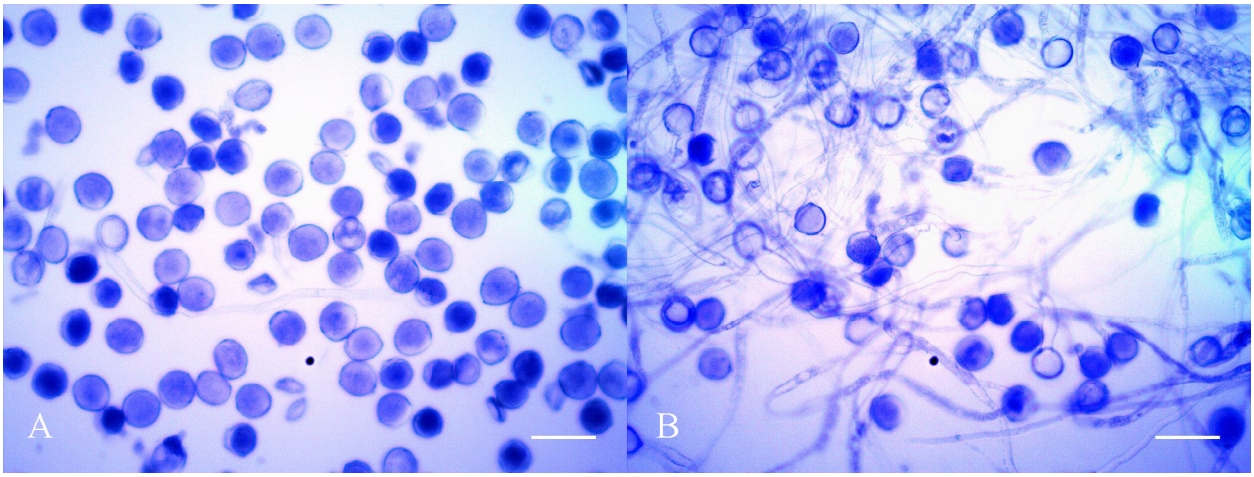

Figure 1: Different stages of *in vitro* pollen germination of Fiji green dwarf coconut. (A) Non-germinated pollen grains. (B) Pollen tube protrusion after 24 h incubation period at room temperature (28 ºC) in Lora culture medium. Bar = 50 μ.

reported variations for *in vitro* pollen germination and pollen tube growth of coconut cultivars (Hebbar et al., 2018). In general, tall cultivars, Chowghat Orange Dwarf (COD) and hybrids showed better adaptability to high temperature while Malaysian yellow dwarf coconut (MYD) was the least adaptable.

Table 3: Percentage of *in vitro* pollen germination, non-germinated and damaged of Fiji green dwarf coconut under different light conditions and incubation periods.

Light conditions	Germination (%)		
	Incubation periods (h)		
	6	24	Mean
Light	11.91	42.90	27.41 a
Dark	10.08	30.90	20.49 a
Mean	11.00 B	36.90 A	
VC (%)	35.21		
	Non-germinated (%)		
Light	60.21	41.20	50.71 a
Dark	61.93	48.77	55.35 a
Mean	61.07 A	44.99 B	
VC (%)	11.75		
	Damaged (%)		
Light	26.74	15.71	21.23 a
Dark	25.83	22.12	23.98 a
Mean	26.29 B	18.92 A	
VC (%)	27.51		

zMeans followed by the same upper-case letter in rows and lower-case letter in columns are not significantly different from each other by Tukey's test at 5% probability.

In vitro germination percentage improved as the sucrose concentration increased (Figure 2a), according to a linear regression (y = 1.3175x - 4.09**), with a coefficient of determination (R^2) 0.87. The highest germination (20.90%) was detected in culture medium supplemented by 16% sucrose. Damage pollen grain percentage reduced as the sucrose concentration increased, according to a quadratic regression (y = -0,1666**x^2 + 2,2738**x + 22,2**), with a coefficient of determination (R^2) 0.998 (Figure 2b). Regression models revealed that there were no significant differences among the *in vitro* non-germination of pollen with respect to sucrose that ranged from 62.32 to 68.04% (data no showed).

The highest *in vitro* pollen germination percentage of five date palm (*Phoenix dactylifera* L.) cultivars was noted by Oliveira et al. (2021) in Marquard medium with 15% of sucrose. In an opposite Soliman, Al-Saif and Al-Obeed, (2016) found the best pollen germination of some male Kadary date palm cultivar in a medium with 8% sucrose. The authors reported that 10% sucrose reduced the pollen germination.

The sucrose is essential to aid in the osmotic process between environment and nutrition for *in vitro* germination (Yaseen et al., 2013). In addition, lack of sucrose has been shown to result in damage to pollen grains under *in vitro* conditions (Baloch et al., 2001). Sugars play a fundamental role in the culture medium and several studies point to sucrose as a stimulating agent for pollen germination (Sousa; Schemberg; Aguiar, 2010). Dafni (1992) points out that the ideal concentrations of sucrose in germination tests for the different pollen types should be in the range of 0 and 500 g L^{-1}.

This report presented the first relevant information for *in vitro* pollen germination of Fiji dwarf coconut. Pollen viability tests represent one of the best options to select the potential of the pollen grain to be used for additional germination (Sakhanokho; Rajasekaran, 2010).

There is a need for complementary studies such as dehydration conditions and maturation stage to increase the viability of Fiji green dwarf coconut pollen grain for the future cryopreservation and breeding programs.

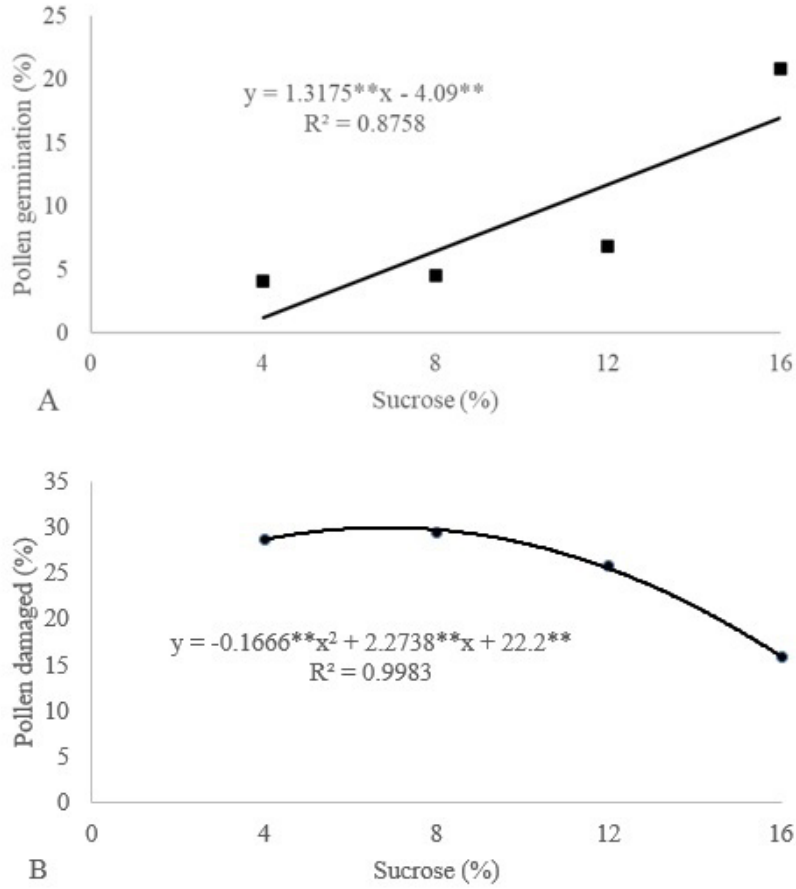

Figure 2: Effect of sucrose concentration on the pollen grain germination (A) and pollen grain damaged (B) of Fiji green dwarf coconut.

CONCLUSIONS

The highest *in vitro* germination of Fiji dwarf coconut pollen was obtained in Lora culture medium by 24 h incubated period at 28 ºC. The light condition does not influence the germination process. *In vitro* germination percentage improved, and damage pollen grain percentage reduced as the sucrose concentration increased.

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DECLARATION OF CONFLICTING INTERESTS

We have no conflict of interest to declare.

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