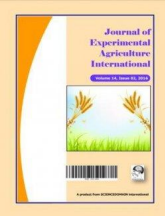

Journal of Experimental Agriculture International

32(1): 1-7, 2019; Article no.JEAI.47221
ISSN: 2457-0591
(Past name: American Journal of Experimental Agriculture, Past ISSN: 2231-0606)

Pollen Viability in Eggplant Using Colorimetric and *In vitro* Techniques

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Authors' contributions

This work was carried out in collaboration among all authors. Authors RNV and DM planned and conducted the study, performed the statistical analysis and wrote the first draft of the manuscript. Authors RNV, LBL, DAN, JASS, AQM, IJNC and DM analyzed and interpreted results. All authors read and approved the final manuscript with the suggestions of the editors

Article Information

DOI: 10.9734/JEAI/2019/v32i130095
Editor(s):
(1) Dr. Mariusz Cycon, Professor, Department and Institute of Microbiology and Virology, School of Pharmacy, Division of Laboratory Medicine, Medical University of Silesia, Poland.
Reviewers:
(1) Moses Oghenenyoreme Eyankware, Ebonyi State University, Nigeria.
(2) Jin Seop Bak, Kyonggi University, South Korea.
Complete Peer review History: http://www.sdiarticle3.com/review-history/47221

Short Research Article

Received 03 December 2018
Accepted 19 February 2019
Published 12 March 2019

ABSTRACT

The causes of fruit abortion, in eggplants, may be related to the absence of viable pollen under high temperatures, common in the Northeast region of Brazil, resulting in a reduction in the number of fruits per plant and consequently in productivity. The objective of this study was to evaluate the techniques of identification of PV – pollen viability in eggplant, as well as to correlate these results with the FFI – Fruit Fixation Index, NFP – Number of Fruits per Plant and PP – Production per Plant obtained in cultivation under high temperatures. The experiment was conducted at the Federal Rural University of Pernambuco, Recife, Brazil, between September and December 2017. The experiment design was a randomized block design with four replications, in the 7 x 4 factorial scheme (7 genotypes x 4 evaluation of PV) containing four plants per experimental plot. The results

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showed that the different techniques used detected viable pollens in the genotypes evaluated in smaller and in larger proportions. The highest percentages were observed in the treatments with CA – Carmine Acetic and AS – Alexander Solution and these overestimated the PV (>90%). In the test with TTC – 2,3,5-triphenyltetrazolium chloride (25%), the viability estimation was equivalent to the in vitro germination test, however the genetic correlations were null and/or very low between these techniques and the FFI, NFP and PP traits. The genetic correlations between the results obtained with CA and AS with FFI, NFP and PP under conditions of high temperatures were high and positive and suggest that the selection based on PV only, except with the use of TTC solution, may be efficient for indirect selection of genotypes with high FFI, NFP and PP.

Keywords: Solanum melongena L; high temperatures; pollen viability; genetic correlations.

1. INTRODUCTION

Eggplant (*Solanum melongena* L.) is an autogamous herbaceous plant, belonging to the Solanaceae family [1]. In Brazil, the area occupied with eggplant is around 1550 ha/year, mainly concentrated in the center-south of the country [2]. In the Northeast of Brazil, the productivity of eggplant cultivation has been unpredictable, especially when flowering coincides with warmer periods of the year, increasing the occurrence of malformation and/or fruit abortion especially when grown under greenhouse conditions. internal temperatures are higher in relation to the external side, causing a considerable reduction of crop yield in the region [3].

The cause of fruit abortion is the absence of viable pollens, because under high temperatures the amount of pollen is drastically reduced, determining a lower number of fruits per plant and consequently lower productivity under such conditions [4]. Specifically for eggplant, the optimal temperature range for fruit growth and production is between 22 and 32°C [5]. Flower abortion is favored by the natural reduction of daylight and by the high temperature of the night (30°C) [6] and productivity is drastically reduced with temperatures above 32°C [5].

Different techniques are cited in the literature to investigate the occurrence of viable pollens in several species and these are grouped in direct methods, such as in vitro germination [7,8,9] and in vivo [9,10,11] or indirect based in cytological parameters such as staining [9,11, 12,13].

Of the different colorimetric techniques adopted, the Carmine Acetic and Alexander Solution reflect only the integrity of cellular structures, such as nucleus and plasma membrane [14,15]. While the 2,3,5-triphenyltetrazolium chloride (TTC) test reflects the activity of dehydrogenase enzymes involved in the respiratory activity of living tissues, whose enzymatic activity of the pollen grain is associated with its germination capacity [16,17].

In vitro germination uses culture media to determine the capacity of pollen grains to develop the pollen tube [18]. However, the success of this technique The success of in vitro germination depends on several factors such as plant species, nutritional state of the plants, time of year and harvest time, photoperiod, temperature, harvest method, incubation period and presence of micro and macronutrients in the culture medium [19], in addition to adjustments of the composition of culture media for each species [20,21].

There is no description of a pollen viability assessment technique with the use of a specific, universal staining dye. However, most studies report the use of nuclear staining dyes, mainly Carmine Acetic, for various groups of plants [22]. Likewise, there is no universal technique for evaluating pollen viability in eggplant. However, few studies with 2,3,5-triphenyltetrazolium chloride staining and in vitro germination are found in the literature [7,8,12].

The aim of this work was to estimate the pollen viability in eggplant genotypes through colorimetric and in vitro techniques, as well as to correlate the results obtained in the laboratory with the results obtained in the greenhouse for agronomic traits and tolerance to high temperatures.

2. MATERIALS AND METHODS

The experiment was carried out in a greenhouse and the pollen viability analyzes carried out at the Plant Production Laboratory, both located in the Department of Agronomy, Planting Area of the Universidade Federal Rural de Pernambuco,

Recife, Pernambuco, Brazil, between September and December 2017.

The statistical design adopted was a Randomized Complete Block design with four replications, in the 7 x 4 factorial scheme (7 genotypes as a function of the 4 different techniques of pollen viability analysis: CA - Carmine Acetic, AS - Alexander Solution, TTC - solution of 2,3,5-Triphenyltetrazolium chloride (25%) and in vitro germination).

The experimental plots consisted of four plants in which, at the peak of flowering, flowers were collected at anthesis, followed by the extraction of the pollen grains with the aid of a portable vibrator and storing them in microtubes with a capacity of 1,5 mL.

In colorimetric techniques, pollen grains were observed 10 minutes after the addition of the staining dyes, except for the staining with 2,3,5-triphenyltetrazolium chloride solution, where the pollen grains were examined after 24 hours. For in vitro germination, the slides were observed 24 hours after preparation with two drops of solution containing 7.5 g of sucrose C12H22O11, 500 mg of calcium nitrate Ca(NO3)2.4H2O, 120 mg of magnesium sulfate MgSO4. 7H2O, 100 mg of potassium nitrate KNO3, 120 mg of boric acid H3BO3 and 10 g of Phytagel™, dissolved in 1000 mL of distilled water.

The pollen grains were observed and photographed under an optical microscope with a 10x objective lens and the pollen viability calculated by the ratio of the number of pollens stained or germinated from 250 pollen per plot. The data were submitted to analysis of variance and the means were compared by the Tukey test at 5% probability.

The PV data were correlated with the agronomic and tolerance traits at high temperatures (FFI – fruit Fixation Index, NFP – Number of Fruits per Plant and PP – Production per Plant) referring to the plants of the plots from where the pollen was collected.The phenotypic (rF), genotypic (rG) and environmental (rE) correlation coefficients were estimated using the Genes program [23]. In addition, we verified the magnitudes of the correlation coefficients, being: r = 0 (null); 0 <|r| <0.30 (weak); 0.30 <|r| <0.60 (average); 0.60 <|r| <0.90 (strong); 0.90 <| r | <1 (very strong) and |r| = 1 (perfect) [24].

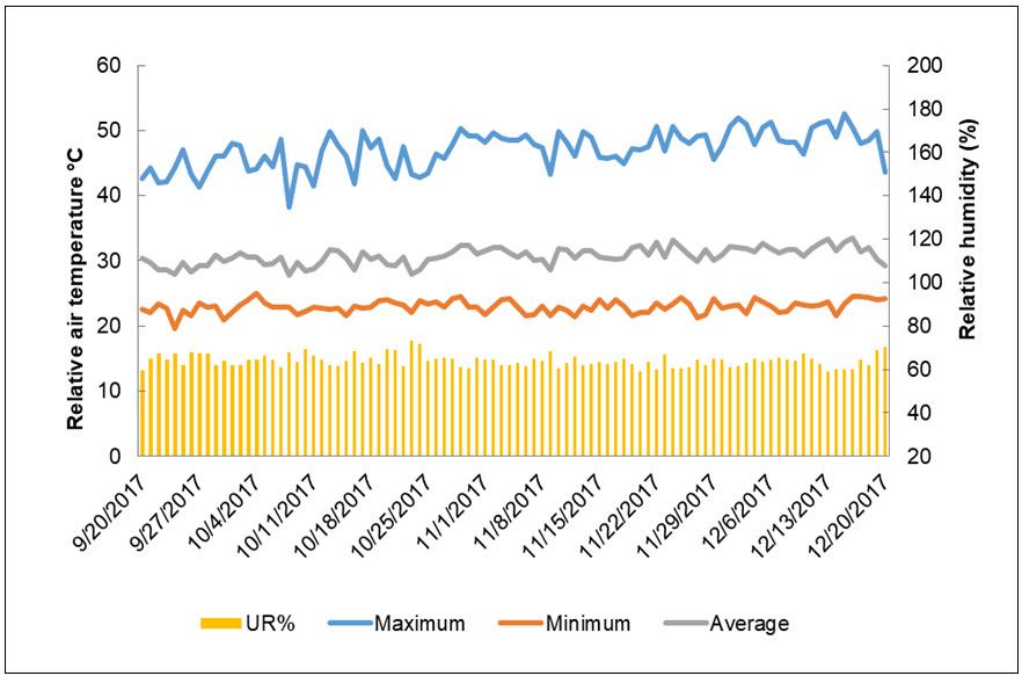

Fig. 1. Relative air temperature (maximum, minimum and average) in the greenhouse between september and december 2017

3. RESULTS AND DISCUSSION

The results of the analysis of variance showed that there were significant differences ($p < 0.1$) in the genotypes regarding the percentages of viable pollen, the efficiency of the techniques adopted and the performance of the genotypes for PV when confronted with the different techniques used. These results suggest the possibility of identifying efficient pollen viability analysis techniques in eggplants, as well as the need for an isolated interpretation of each factor due to the observed GxT interaction (Table 1).

Regarding the PV techniques used, it was verified that in the CA-treated pollen the percentage of viable pollen grains, stained with intense red was 94.2%, indicating the integrity of the chromatin, a result that did not differ from that obtained by the AS test, in which the results show that 92.1% of the pollen grains are viable, because they have intact protoplasm and cell wall, indicated by the pink color of the protoplasm and a fine green outline of the cell wall (Table 1). There are no reports in the literature of the use of CA and AS in the identification of pollen viability of eggplants, however, these dyes overestimate pollen viability (> 90%), but are efficient in determining cell constituents and integrity of the pollen grain [16].

The TTC test that reflects the presence of active dehydrogenases enzymes was the only colorimetric technique that provided a statistically similar result to the in vitro germination test. It is considered a reliable estimate, close to that provided by in vitro germination tests [25]. The TTC test proved to be a reliable and fast tool, providing an average estimate of 38.8% viable pollen (Table 1). However, the results for the genotypes CNPH 47, CNPH 109, CNPH 51, CNPH 60 were superior to the 42.8% of maximum viability obtained by other authors for the same TTC concentration (25%) [12]. On the other hand, other authors reported that the TTC staining technique was not efficient for pollen evaluation of Ciça F1 eggplant due to the difficulty of the pollen grains coming in contact with the TTC solution [8].

Although the histochemical test is quick, easy and cheap, it should not be the only method used to estimate the viability of pollen since it does not provide information on germination capacity. This information can be obtained by in vitro and in vivo germination tests [26]. Although some authors have reservations to the use of TTC, stating that it can provide ambiguous results, since aborted pollen grains may have a color similar to that of viable grains [27].

The germination of the pollen grains by the in vitro technique was 41.8% (Table 1). The genotypes presented averages between 13.5 and 69.9%. Highlighting the genotypes CNPH 141 (69.9%) and CNPH 60 (60.8%). Other authors using the in vitro technique reported a maximum pollen germination of 10.80% for the Ciça F1 eggplant, and it was necessary to calibrate the culture medium to obtain more satisfactory results. On the other hand, other authors indicated that the in vitro method used for another eggplant cultivar is reliable to quantify the viability of the pollen, these authors obtained after eight hours of germination percentage incubation of 79% [7].

Pollen viability was detected regardless of the dye used, however, the only percentages of viable pollens higher than 80% were in the treatments with CA and AS, and these dyes could have overestimated the values of viable pollens as reported by other authors in different species [28,29]. Colorimetric techniques overestimate pollen viability, while the in vitro test underestimates it [30]. This was observed when comparing in vitro germination values with colorimetric techniques, thus the need to correlate with some other technique [22] (Table 2).

Although coloring is a simple procedure, it is not totally reliable because, as previously reported, it may provide misleading information on viability [22]. In addition, in vitro germination, while providing a controlled experimental system, does not completely reproduce pollen tube growth in vivo, and interactions between the culture medium composition and the different plant materials may occur [31].

Estimates of phenotypic and genetic correlations between the results of pollen viability obtained by the different colorimetric and in vitro techniques and the results for FFI, NFP and PP showed similarity in the direction and magnitude of the correlations (Table 2). Even though phenotypic correlations have genetic and environmental causes, only the genetic ones involve an association of inheritable nature [32] and therefore, they are more important in the selection process of genotypes.

Estimates of positive genetic correlations and above 0.6 were obtained in about 67% of the

pairs obtained (Table 2). However, in the correlations between the pollen viability obtained by TTC staining and the agronomic and tolerance traits at high temperatures, the correlations were negative and/or of low magnitude [24]. These results indicate that the selection of genotypes based on PV, except with the use of TTC solution, could result indirectly in the increase of FFI, NFP and PP (Table 2).

In the estimates of the environmental correlations, the estimates were mostly negative and of very low magnitudes in 91.7% of the pairs obtained, except for the pair CA x PP (0.8) (Table 2). Although, the environmental correlations in the pair CA x PP (0.8) were high and positive. Environmental correlations occur between two traits when they are influenced by the same variations of the environment. When negatives indicate that the environment favors one character to the detriment of the other and when positive, indicates that both traits were benefited or harmed by the same environmental causes [32].

Table 1. Pollen viability (PV) for eggplant genotypes obtained by colorimetric and in vitro techniques

Genotypes	Pollen viability (%)			
	CA	**AS**	**TTC (25%)**	***In vitro***
CNPH 135	96.6 Aa	93.5 Aab	24.0 Ccd	46.7 Bbc
CNPH 47	90.2 Aa	80.5 Ab	53.8 Ba	13.5 Cf
CNPH 109	97.5 Aa	96.1 Aa	44.5 Bab	46.9 Bbc
CNPH 51	97.7 Aa	94.4 Aab	49.3 Bab	44.9 Bcd
CNPH 60	95.4 Aa	95.2 Aab	45.4 Cab	60.8 Bab
CNPH 53	82.9 Aa	86.3 Aab	37.8 Bbc	20.7 Ccf
CNPH 141	97.8 Aa	95.1 Aab	40.0 Cab	69.9 Ba
Ciça $_{F1}$	95.4 Aa	95.6 Aab	15.6 Cd	31.2 Bde
Means	94.2 A	92.1 A	38.8 B	41.8 B
$QM_{(Genotypes)}$	876.7**			
$QM_{(techniques)}$	29824.5**			
$QM_{(GxT)}$	498.5**			
Overall average	66.7			
$CV_{(\%)}$	10.6			

Means followed by the same capital letters in HORIZONTAL do not differ statistically from each other by the Tukey test at 5% probability; Means followed by the same lowercase letters in VERTICAL do not differ statistically from each other by the Tukey test at 5% probability

Table 2. Genotypic correlation coefficients (rG), phenotypic (rf) and environmental (rE) between traits evaluated in eggplant

Characters		FFI	NFP	PP
CA	r_F [1]	0.6	0.7*	0.9**
	r_G [2]	0.7	0.8	1.0**
	r_E [2]	0.1	0.1	0.8
AS	r_F [1]	0.2	0.5	0.6
	r_G [2]	0.4	0.6	0.9
	r_E [2]	-0.3	-0.1	-0.1
ST	r_F [1]	0.1	0,0	-0.3
	r_G [2]	0.1	0.0	-0.4
	r_E [2]	0.1	0.0	0.1
In vitro	r_F [1]	0.5	0.6	0.5
	r_G [2]	0.7	0.7	0.6
	r_E [2]	-0.1	-0.2	-0.2

** and ** significant at 1 and 5% probability by the F test respectively.*
** and ** Significant at 1% and 5% probability by test t[1].*
** and ** Significant at 1 and 5% probability by the bootstrap method with 5000 simulations[2]*

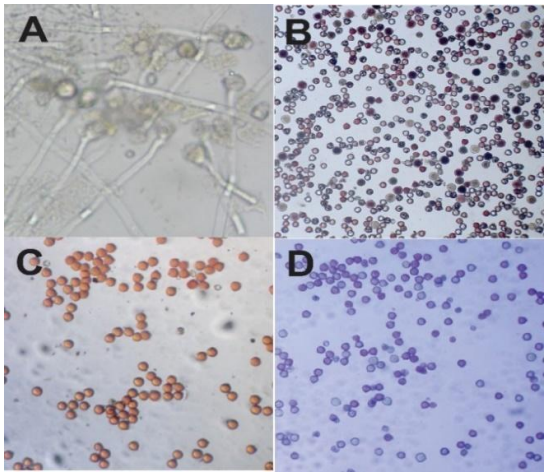

Fig. 2. Viable eggplant pollen grains obtained by colorimetric and in vitro techniques

(A) in vitro germination, (B) Solution of 2,3,5-triphenyltetrazolium chloride (25%), (C) Carmine Acetic, (D) alexander Solution

4. CONCLUSION

The results showed that the different techniques used detected viable pollens in the genotypes evaluated in smaller and in larger proportions. The highest percentages were observed in the treatments with CA – Carmine Acetic and AS – Alexander Solution and these overestimated the PV (>90%). In the test with TTC – 2,3,5-triphenyltetrazolium chloride (25%), the viability estimation was equivalent to the in vitro germination test, however the genetic correlations were null and/or very low between these techniques and the FFI, NFP and PP traits. The genetic correlations between the results obtained with CA and AS with FFI, NFP and PP under conditions of high temperatures were high and positive and suggest that the selection based on PV only, except with the use of TTC solution, may be efficient for indirect selection of genotypes with high FFI, NFP and PP.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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