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Pitaya genotypes (*Hylocereus* spp.) seed germination at different pH levels based on statistical models

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Abstract

Seed germination depends on internal and external factors relating to the seed but the whole process will normally occur if there are no restrictions during the stages of germination. So, the aim of the present study was to evaluate the viability and the vigor of pitaya seed under different pH levels. A completely randomized design was employed under a 3 × 13 factorial design [pitaya genotypes: *Hylocereus undatus* (white pitaya); *H. undatus* x *H. costaricensis* (pitaya hybrid I), and *H. costaricensis* x *H. undatus* (pitaya hybrid II), and pH levels: 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, and 9.0], with four replications. The solutions of pH levels were prepared with distilled water added by hydrochloric acid (HCl) and/or sodium hydroxide (NaOH), and measured with the aid of pH meter. In order to conduct the germination test, 50 seeds were used per repetition, arranged in polystyrene boxes (Gerbox® type) lined with blotter paper and moistened with solutions referring to pH. The experiment was conducted in germinators with photoperiod of 12h and constant temperature of 25ºC. The variables evaluated were percentage of germination (PG), germination speed index (GSI), and mean germination time (MGT). The data pertaining to PG were fitted to a almost-binomial model and the data pertaining to GSI and MGT were fitted to linear regression models. *Hylocereus* spp. seeds are sensitive to pH changes of the substrate. The pH levels lower than 4.5 damaged the viability and the vigor of pitaya seed, while the pH range between 6.0 and 7.5 is favorable for the germination process.

Keywords: hybridization; *Hylocereus costaricensis*; *H. undatus*; physiological quality; viability; vigor.
Abbreviations: ºC_degree Celsius; GSI_germination speed index; h_hours; MGT_mean germination time; mm_millimeter; PG_percentage of germination.

Introduction

Cactaceae family (Eudicot) comprises about 200 genera and 2,000 species, from which approximately 80 species have agricultural aptitude (Kiesling, 2001; Areces, 2004; Taylor and Zappi, 2004). Pitaya (*Hylocereus* spp.), known as dragon fruit due to its scales, is the denomination used for both the plant and the fruit. It originated in tropical and subtropical America (Le Bellec et al., 2006). This species has occupied a growing niche in the exotic fruit market, arousing the interest of the consumers, due to their organoleptic characteristics and nutraceutical properties (Silva et al., 2006; Andrade et al., 2007; Gunasena et al., 2007) and of the fruit producers, regarding to the aggregate commercial value (Bastos et al., 2006). The fruit is considered nutritious and can be consumed both in natura and processed in a range of industrialized products. The pulp is succulent, where numerous seeds are distributed. The seeds are obovate-shaped, dark, smooth, and bright, with approximately 3 mm in diameter (Le Bellec et al., 2006; Lorenzi et al., 2006).

Propagation can be performed by means of seeds or vegetative structures. The seeds present high germination capacity and less possibility of transmitting diseases (Verheij, 2005; Elobeidy, 2006), being convenient to obtain different genetic information. Its diversity can be used in the selection of genotypes with desirable characteristics such as productivity, external appearance, pulp coloring and better adaptation to different climatic conditions (Andrade et al., 2008).

The germination percentage depends on internal and external factors related to the seeds. Thus, the whole process will normally occur if there is no restriction during the germination stages (Wagner Júnior et al., 2007). It is known that several factors are involved and are likely to influence seed germination, being that the most studied are water, oxygen, temperature, light, and substrate. Besides these, pH is also a factor of relevance for the germination process and development of seedlings of cultivated species, although is relatively little studied (Ortiz et al., 2015).

According to the Rules for Seed Analysis (Brasil, 2009), for most species, it is recommended that the pH of both the substrate and the water should be between 6.0 and 7.5. However, there are no data for cacti. The International Seed Testing Association (Ista, 2015) have also recommended this pH range (between 6.0 and 7.5), which, according to Kerbauy (2012), favors the germination process of most plant species.

The influence of pH on germination has received little attention. From the few studies considering the pH effect on the germination process, it has been observed that pH levels between 3.0 and 11.0 in *Lactuca sativa* (Wandscheer et al., 2011) and between 3.0 and 9.0 in *Hylocereus* spp. (Ortiz et al., 2015) has not significantly influenced the percentage of germination. However, Ortiz et al. (2015) found a significant effect of pH on vigor, expressed by GSI, in one of the three genotypes of *Hylocereus* spp. evaluated, with lower performance at pH 4.0; 5.0 and 6.0.

Differential effects depending on pH levels can be observed according to the evaluated genetic material. Due to a few researches related to pitaya, the need for studies is emphasized to obtain information that can be applied to crops under world soil and climatic conditions (Cavalcante et al., 2011). Thus, the aim of this study was to evaluate the viability and the vigor of pitaya seeds under different pH levels.

Results and Discussion

Percentage of germination of pitaya genotypes seeds at pH levels

Figure 1 shows the percentage of germination (PG) of pitaya genotypes seeds submitted to different pH levels, where the interaction between the factors (genotype and pH) and the quadratic term are significant. For white pitaya, pH levels between 5.5 and 7.5 favored the PG. Similar behavior was observed for the seeds of the pitaya hybrid I and pitaya hybrid II, which had the germination process optimized when submitted to the pH levels of 6.0 to 8.0, expressing greater PG in this interval. However, according to Ortiz et al. (2015), the pH effect was not significant for seed germination of three genotypes of *Hylocereus* spp.

The lowest performances occurred in acid pH levels (lower than pH 4.5); pH levels between 6.0 and 7.5 were favorable to the germination process of *Hylocereus* spp. seeds, in order to provide satisfactory conditions for the germination of the three evaluated genotypes (Fig. 1). The results found in the present study confirm the recommendations from the Rules for Seed Analysis (Brasil, 2009) and the International Seed Testing Association (Ista, 2015). Both recommend the germination of most species at pH between 6.0 and 7.5.

Similar results were obtained by Evenari (1949) when studying seeds of *Lycopersicon* esculentum and *Malus* spp., and by Ferreira (1976) on *Mimosa bimucronata*, verifying that the seeds of these species do not germinate in acidic environment.

According to Wandscheer et al. (2011) in *L. sativa* and Therios (1982) in *Prunus amygdalus* seeds, the broad pH ranges of 3.0 to 11.0 and between 2.8 and 8.4, respectively, were not able to significantly influence the percentage of germination of these species.

Germination speed index and mean germination time of pitaya genotypes seeds at pH levels

Regarding to the variables of the germination speed index (GSI) and mean germination time (MGT), it is noteworthy that they were not measured at the pH levels 3.0 and 3.5 for white pitaya and at 3.0 for pitaya hybrid II, due to the lack of germinated seeds of these genotypes under these conditions. In addition, as found for PG, when analyzing GSI and MGT it was also found that both the interaction between the factors (genotype and pH) and the quadratic term were significant (Fig. 2 and 3).

With regard to GSI, it was found that pH levels between 5.5 and 7.5 for white pitaya once again contributed to the germination process, as well as observed when evaluating the PG. For the pitaya hybrid I and pitaya hybrid II, the pH levels between 6.0 and 8.0 provided the highest GSIs. The lowest indexes were obtained in acid pH ranges, lower than 4.5. The levels of pH 6.0 to 7.5 were those that provided higher GSI in the *Hylocereus* spp. genotypes evaluated (Figure 2).

Since germination and seedling development can be influenced in extremely acid or alkaline medium, a pH level between 6.0 and 7.5 is recommended, which is ideal for the germination of most plant species (Kerbauy, 2012) and collaborates on biochemical processes and plant nutrition (Larcher, 2000).

Figure 3 shows the behavior of pitaya genotypes seeds submitted to different pH levels for MGT. For white pitaya, the MGT did not differ significantly at pH levels, but differing from the results observed by Ortiz et al. (2015). In the case of the pitaya hybrid I and pitaya hybrid II, it was observed that the MGT decreased with as the pH increased, which is advantageous. This confirms once again that a pH level of extreme acidity (less than 4.5) was unfavorable to the seeds of these genotypes, because it caused a delay in the germination process, since it demands more time in average for the seeds to germinate.

Final considerations

The performance of the pitaya hybrids was highlighted in the different pH levels in relation to their paternal, on PG, GSI and MGT, and can be justified by the heterosis. These results corroborate with the research of Coimbra et al. (2006), who concluded that the heterosis in hybrid rice cultivars is one of the most important technical applications of genetics in agriculture, obtaining more vigorous and higher yielding cultivars.

Thus, it is observed that the pH factor influences the viability and the vigor of *Hylocereus* spp. seeds. The results obtained in this study confirm the recommendations as per Brasil (2009) and Ista (2015), where pH levels close to neutrality (6.0-7.5) are favorable to the germination process, reducing its efficiency when tending to acidity and/or alkalinity. This fact was confirmed by the trend presented in the three *Hylocereus* spp. genotypes evaluated.

However, because each genetic material presents a peculiar response, it is necessary to evaluate the performance of

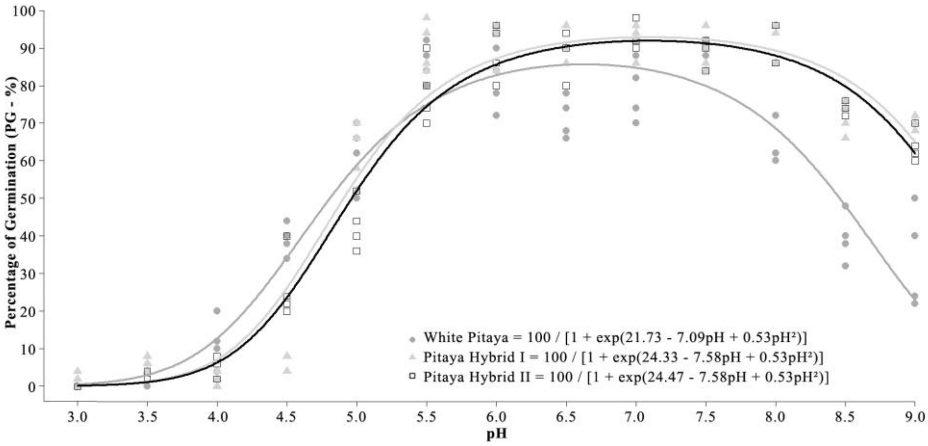

Fig 1. Adjust of the almost-binomial regression model for Percentage of Germination (PG - %) of three pitaya genotypes seeds submitted to thirteen pH levels.

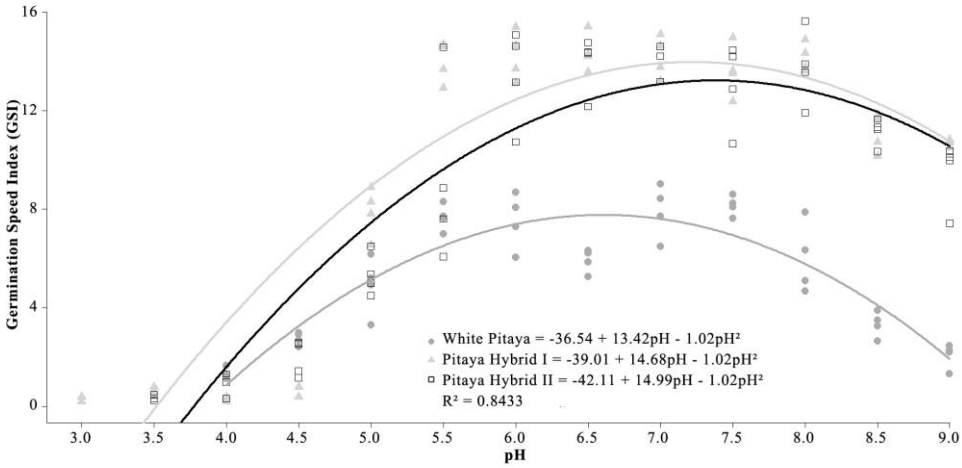

Fig 2. Adjust of the linear regression model for Germination Speed Index (GSI) of three pitaya genotypes seeds submitted to thirteen pH levels.

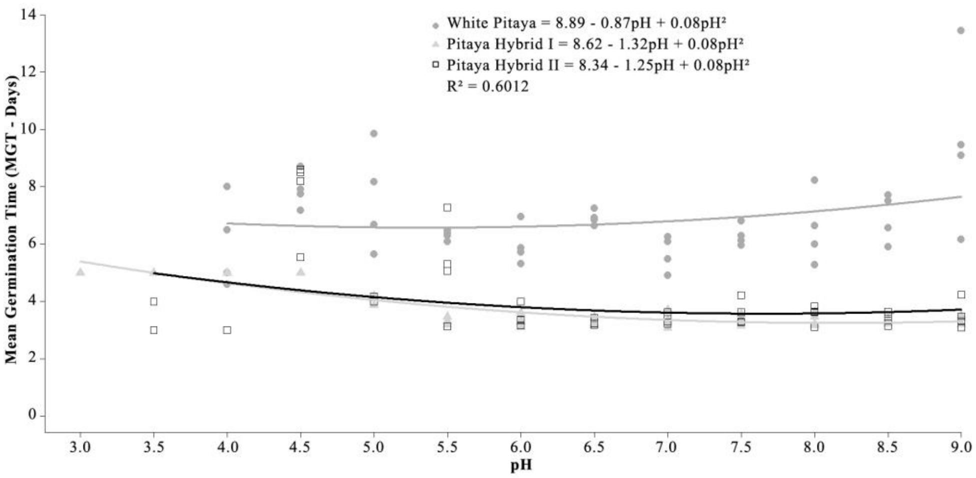

Fig 3. Adjust of the linear regression model for Mean Germination Time (MGT - days) of three pitaya genotypes seeds submitted to thirteen pH levels.

each one according to the pH levels. With that said, due to the scarcity of agronomic information about the management and cultivation of pitaya, as well as scientific basics related to the effect of pH on seed germination and seedling development, new research should be carried out to optimize the production of this species and increase the area in the world scenario.

Materials and Methods

Study area characterization and plant material

This study was conducted in the Seed Production and Technology Laboratory at the State University of Londrina (Universidade Estadual de Londrina - UEL), Londrina, Brazil. Seeds were obtained from ripe fruit of parent plants of *Hylocereus undatus* (white pitaya), and the hybrids *H. undatus* x *H. costaricensis* (pitaya hybrid I) and *H. costaricensis* x *H. undatus* (pitaya hybrid II), cultivated in the experimental area of the Department of Agronomy at UEL, which is located at 23º 23' S and 51º 11' W at a mean altitude of 566 m.

Pitaya plants, aged for approximately 10 years, were grown in soil area classified as RED NITISOL Eutroferric latosol (Embrapa, 2013), planted every 2.0 × 3.0 m, and supported by 2.5-m-high sticks with two plants per stick.

Seed extraction

Pulp of the fruit was extracted manually using a spoon and placed in a 2 L beaker with a solution consisting of water (1 L) and sugar (25 g L^{-1}). Then, this mixture was allowed to rest for 48 h at room temperature to favor the fermentation process and facilitate seed extraction. After the resting period, the solution was sieved under running water to eliminate pulp residues and retain the seeds, which were subsequently placed on filter paper and dried in the shade at room temperature for 48 h.

Treatments

The experimental design was completely randomized, under a 3 × 13 factorial design corresponding to the 3 pitaya genotypes [*Hylocereus undatus* (white pitaya), *H. undatus* x *H. costaricensis* (pitaya hybrid I), and *H. costaricensis* x *H. undatus* (pitaya hybrid II)] and 13 pH levels (3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, and 9.0), with four replications.

To obtain the pH levels, solutions were prepared with distilled water, adding hydrochloric acid (HCl) and/or sodium hydroxide (NaOH) and measured using a Jenway® pH meter, model 3510. A sufficient quantity of each solution was prepared to set up and conduct the experiment, as well as to re-wet the blotting paper if the initial material evaporated. The substrate was moistened according to the variation in its absorption capacity – that is, until saturation, without excess.

Measured variables

For the analysis of the percentage of germination (PG), germination speed index (GSI) and mean germination time (MGT), 50 seeds were used per repetition, arranged in polystyrene boxes (Gerbox® type) lined with blotter paper, moistened with solutions referring to pH.

The experiment was conducted in germinators with photoperiod of 12 h and constant temperature of 25 ºC. The evaluations were performed daily for 30 days, when the germination stabilized. The botanical concept of germination was adopted – that is, the seeds that presented root extension equal to or greater than 2 mm were been considered germinated. The results were expressed as percentage of germinated seeds. In addition to the germination test, GSI was determined according to the methodology of Maguire (1962) and MGT in days, according to Lima et al. (2006), from daily counts of germinated seeds.

Statistical analysis

Statistical analysis were performed using software R (R Development Core Team, 2017). To verify the germination potential of the pitaya genotypes in relation to the pH levels, the almost-binomial model was adjusted, in which the response variable is the percentage of germinated seeds. To check the fit of the model, a semi-normal probability graph with a simulation envelope was constructed. From this verification, we found evidence of the model providing a good fit, since most of the points were within the envelope (Urbano et al., 2013). Models were made for the GSI and MGT variables adjustments of linear regression, in which the explanatory variable was the pH level.

Conclusion

Hylocereus spp. seeds are sensitive to pH changes of the substrate. The pH levels lower than 4.5 damaged the viability and the vigor of pitaya genotypes seeds, while the pH range between 6.0 and 7.5 is favorable for the germination process.

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