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Effect of fruits maturation stage and vegetal regulators in the physiological quality of seeds sweet passion fruit cv. BRS Mel do Cerrado

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Abstract: This study aimed to evaluate the effect of fruit maturation stages and pre-germination treatments with plant regulators on the physiological quality of seeds of cv. BRS Mel do Cerrado. Two experiments were carried out, germination and emergence, installed in a completely randomized design in a 3 x 2 factorial arrangement, with three stages maturation (1-partially ripe fruits; 2-fully ripe fruits and 3-senescent fruits) and two pre-germination treatments (1. [GA_{4+7} +N-(phenylmethyl)-aminopurine (300 ppm)] and 2. distilled water), forming six treatments with four replications of 50 seeds. The evaluated characteristics were germination percentage at 14 and 35 days, germination speed index (GSI), emergence percentage and emergence speed index (ESI). Germination at 14 days, GSI and seedling emergence were not affected by the maturation stage, however germination and GSI were higher when the plant regulator was used. The final percentage of germination was higher in seeds taken from partially ripe fruits and treated with regulators and a higher ESI was observed for seeds from senescent fruits and treated with regulator. Seeds treated with plant regulators are more vigorous and have a higher rate of seedling emergence.

Index terms: *Passiflora alata* Curtis, physiological maturity, pre-germination treatment, seed vigor, seminiferous propagation.

Efeito do estádio de maturação de frutos e reguladores vegetais na qualidade fisiológica de sementes de Maracujá Doce

Resumo: Este trabalho teve como objetivo avaliar o efeito de estádios de maturação de frutos e de tratamentos pré-germinativos com reguladores vegetais na qualidade fisiológica das sementes de maracujá doce cv. BRS Mel do Cerrado. Foram realizados dois experimentos, de germinação e de emergência, instalados em deli-

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neamento inteiramente casualizado, em arranjo fatorial 3 x 2, sendo três estádios de maturação (1-frutos parcialmente maduros; 2-frutos totalmente maduros, e 3-frutos senescentes) e dois tratamentos pré-germinativos (1. [GA_{4+7} +N-(fenilmetil)-aminopurina (300 ppm)] e 2. água destilada), formando seis tratamentos com quatro repetições de 50 sementes. As características avaliadas foram percentagem de germinação, aos 14 e 35 dias, índice de velocidade de germinação (IVG), percentagem de emergência e índice de velocidade de emergência (IVE). A germinação, aos 14 dias, o IVG e a emergência de plântulas não foram afetados pelos estádios de maturação; entretanto, a germinação e o IVG foram maiores quando se utilizaram os reguladores vegetais. A porcentagem final de germinação foi maior para sementes retiradas de frutos parcialmente maduros e tratadas com reguladores vegetais e observou-se maior IVE para sementes de frutos senescentes e tratadas com reguladores. Sementes tratadas com reguladores vegetais são mais vigorosas e apresentam maior velocidade de emergência de plântulas.

Termos para indexação: *Passiflora alata* Curtis, maturidade fisiológica, tratamento pré-germinativo, vigor de sementes, propagação seminífera.

Introduction

Sweet passion fruit (*Passiflora alata* Curtis) is a species native to South America, being widely found in the Brazilian territory and as part of domestic and commercial crops (FALEIRO et al., 2017; CARLOSAMA et al., 2020). Its fruits are mainly sold in fresh fruit markets, and it can be consumed *in natura* or used as an ingredient in several recipes. All parts of the passion fruit can be used as an ingredient in different recipes including its peel (FALEIRO et al., 2021).

Passion fruits are also a source of active principles for the pharmaceutical and cosmetic industries since its constitution includes bioactive phyto components with substances responsible for the anxiolytic, sedative, diuretic, analgesic, and antioxidant properties (COLOMEU et al., 2014; FIGUEIREDO et al., 2016). Because of its beautiful leaves, flowers, and fruits, it can also be used as an ornamental plant in the landscape of big areas composing pergolados, fences, and walls (FALEIRO et al., 2021).

The first cultivar belonging to *P. alata* BRS Mel do Cerrado (BRS MC) species was introduced in 2017 through the Programa de Melhoramento Genético do Maracujazeiro da Embrapa, being registered and protected at the Ministry of Agriculture, Livestock and Supply. The cultivar was developed to solve the increasing demand in the market for special fruits with a high added value. During the improvement stages, selections were performed aiming the more productive plants with fruits showing the highest physical and physicochemical quality and highest level of tolerance to foliar diseases (FALEIRO et al., 2021).

When a cultivar is introduced in the market, farmers start looking even more for a high-quality propagation material. Just like most of the passion fruits, the main propagation route for the cv. BRS MC is through its seedlings. To meet market requirements, the seeds produced and marketed should have physical, genetic, sanitary, and physiological characteristics that ensure a quality standard in the production of seedlings. According to Oliveira et al. (2020), information such as the germination and emergence index and also the evaluation of the seeds vigor is fundamental to enable the production and commercialization logistics and commercialization of passion fruit seeds and seedlings.

The physiological quality of seeds is essential to successfully obtain the seedlings and it is closely associated to the physiological maturity of the seeds, a period when they show the maximum potential of germination and vigor (CARVALHO; NAKAGAWA, 2012). As stated by Negreiros (2006), the maturation stage of *Passiflora* spp. fruits is an important factor that might be associated to the quality of seedlings. Therefore, it is necessary to eval-

uate it to obtain the highest seeds response in terms of germination and vigor. Zucareli et al. (2009) report that vegetable regulators, as gibberellin and cytokinin, have been used as pre-germination treatments in passion fruit seeds. These phytohormones work favoring the germination process of the seeds, triggering metabolic processes (TAIZ e ZEIGER, 2017) that might stimulate and accelerate the emergence process of the seedlings.

Based on what we described above, this study aimed to evaluate the effect of fruit maturation stages and pre-germination treatments with plant regulators on the physiological quality of cv. BRS Mel do Cerrado (BRS MC) seeds.

Material and Methods

The experiments were carried out at Unidade de Apoio da Fruticultura and Setor de Viveiros e Casas de Vegetação da Embrapa Cerrados in Planaltina (Federal District) from January to March, 2022. The fruits were harvested from plants originated from genetic seeds of the cv. BRS MC grown by Embrapa licensee. A visual selection based on the epicarp color of fruits was performed and the fruits were distributed in three groups with different maturation stages: 1 – partially ripe fruits (30-50% of the yellow epicarp); 2- fully ripe fruits (100% of the yellow epicarp) and 3-senescent fruits (fruits of an intense orange color with necrosis). Two pre-germination treatments, one using vegetable regulators [GA_{4+7} +N-(phenylmethyl)-aminopurine (300 ppm)] and the other only using distilled water, were combined to the maturation stages of the fruits.

After separating and washing the fruits, the seeds were manually extracted, and the aryl was removed from the seeds by using the friction method in a wire mesh under current water. After the seeds were cleaned, they were placed in the shade to be dried for 4 days over a paper towel. The seeds were counted and then emerged in their respective pre-germination treatments to be embedded for 30 minutes. Next, the germination and emergence tests were set up according to recommendations by Regras para Análise de Sementes (Rules for Seeds Testing) (BRASIL, 2009).

Germination test

The germination test consisted of having the seeds spread over two Germitest® sheets of paper and moistened with an amount of distilled water equivalent to 2.5 ratio times the non-hydrated paper mass. Subsequently, rolls were made and packed into plastic bags and kept in a germination chamber, type BOD, kept at 25 ºC at night and 30 ºC during the day, a photo period of eleven hours of light.

The seeds were considered germinated when presenting a visible root protrusion and a root protrusion in development. The analyzed variables were the germination percentage at 14 and 35 days after incubation and the germination speed index (GSI) using the formula proposed by Maguire (1962).

Emergence test

Sowing for the emergence test was performed in polyethylene trays with 50 cells filled with a coconut fiber and wood fiber-based commercial substrate. One seed was placed in each cell at a depth of ± 0.5 cm. The experiment was kept in a greenhouse with temperatures ranging from 20 to 30º C and included a daily irrigation consisting of two waterings a day (in the morning and afternoon). The emerged seedling percentage was evaluated every two days up to 35 days after sowing. The variables analyzed were final percentage of seedling emergence and emergence speed rate by using the formula proposed by Maguire (1962). The seedlings were considered emerged when the cotyledons were totally expanded.

Statistics

The experiments were made in completely randomized design in a 3 x 2 factorial arrangement (fruits maturation x pre-germination treatment) consisting of six treatments with four replications of 50 seeds, with a total of 24 experimental parcels. Statistical analysis was performed by checking the normality of errors using the Shapiro-Wilk test and the homogeneity of variances using the Bartlett test. Once the normality and

homogeneity of the data were verified, the analysis of variance was performed, being conclusive for the study of the averages for the pre-germination treatments (vegetable regulators and distilled water) and for the non-significant variables. As for the significant variables, the averages were compared using the Tukey test at 5% significance, using the R statistical software (R DEVELOPMENT CORE TEAM, 2021).

Results and Discussion

The variance analysis showed a significant interaction between the factors for the germination percentage in the second count and emergence speed index (ESI)(ESI). When considering the germination percentage in the first count and the germination speed index (GSI) only the pre-germination treatment was found significant. The variable seedling emergence did not show a significant effect (Table 1).

Table 1. Summary of the analysis of variance for the germination percentage at the first (%G-1C) and second count (%G-2C), germination speed index (GSI), seedling emergence percentage (%E) and emergence speed index (ESI) of seedlings (ESI) for cv. BRS Mel do Cerrado for the fruits maturation stages (FMS) and pre-germination treatments (PGT). Embrapa Cerrados, Planaltina, DF, 2022.

SV	DOF	QMQ				
		%G-1C	%G-2C	GSI	%E	ESI
FMS	2	8.00^{ns}	52.67*	0.02^{ns}	7.17^{ns}	0.16**
PGT	1	32856.00**	45240.17**	94.05**	10.67^{ns}	3.95**
FMS*PGT	2	16.00^{ns}	52.67*	0.02^{ns}	8.17^{ns}	0.13**
Debris	18	376.00	13.17	0.05	13.56	0.01
GM		**37.00**	**44.92**	**2.01**	**93.67**	**2.62**
CV (%)		**12.35**	**8.08**	**11.52**	**3.93**	**3.93**

SV – source of variation, DOF – degree of freedom, MQ – mean square, GM - general mean, CV – coefficient of variation. **Significant ($p<0.001$). *Significant ($p<0.05$). ^{ns} Non significant.

The highest mean germination percentage in the second count was observed among the seeds treated with vegetable regulators and extracted from partially ripe fruits (94%). However, when studying the ESI, the highest mean (3.32) was found in seeds originated from fruits in a senescent stage. The highest germination percentage and ESI were observed in seeds treated with vegetable regulators in all maturation stages of the evaluated fruits (Table 2).

Table 2. Mean of germination percentage of seeds in the second count (%G-2C) and for the emergence speed index (ESI) of seedlings (ESI) of the cv. BRS Mel do Cerrado, for the fruits maturation stages (FMS) and pre-germination treatments with vegetable regulators (VR) and distilled water (AD). Embrapa Cerrados, Planaltina, DF, 2022.

FMS	%G-2C		ESI	
	VR	AD	VR	DA
Partially ripe	94.00 aA	1.50 aB	2.80 bA	2.24 aB
Totally ripe	87.00 bA	1.50 aB	2.96 bA	2.15 aB
Senescent	84.00 bA	1.50 aB	3.32 aA	2.24 aB

The means followed by the same small letters, in the column and capital letters in the line do not show a difference according to Tukey test 5% de probability of error.

The lowest germination percentage in the second count found in seeds obtained from totally ripe fruits and senescent fruits might be related to the focus of contamination by microorganisms in these treatments. since when the emergence test in a commercial substrate was performed no significant difference was noted in the percentage of seedlings emerged in different maturation stages ranging from 93% to 94.75%. It was also noted that in the germination first count at 14 days performed to verify the seed vigor, no significant difference among the averages for the maturation stages was found. This was also true for the GSI (Table 3).

Table 3. Mean of germination percentage in the first count (%G-1C), germination speed index (GSI) and emergence percentage de seedlings (%E) of the cv. BRS Mel do Cerrado for fruits maturation stages (FMS). Embrapa Cerrados, Planaltina, DF, 2022.

FMS	%G-1C	GSI	%E
Partially ripe	37.00 a	2.06 a	94.75 a
Totally ripe	38.00 a	2.02 a	93.00 a
Senescent	36.00 a	1.96 a	93.25 a

Means followed by the same letters in the column do not differ from each other by the F test of the analysis of variance at 5% probability of error.

These results evidenced that in terms of seed propagation of the cv. BRS MC, the fruits might be harvested before being totally ripe until its senescence, with no damage to the seedling emergence in a commercial substrate. The use of vegetable regulators accelerates the seedling emergence providing a more uniform and vigorous seedling stand. Similar to this research, Santos et al. (2016) concluded that the maturation stage of the *Passiflora* spp. fruits, including the *P. alata*, did not influence the percentage of seedling emergence at 60 days after planting the seeds. Besides that, the authors also observed that the recent harvested seeds of *P. alata*, *P. cincinnata*, *P. edulis*, *P. gibertii* and *P.setacea* show a faster emergence when the vegetable regulators are used. On the other hand, Junghans et al. (2012) found out a lower seedling emergence in "almost ripe" fruits for the *P. alata* species. The performance of Passiflora seeds in terms of seed germination and seedling emergence is very variable due to experimental conditions and depending on the species and even the accession or evaluated variety (OLIVEIRA et al., 2020).

When studying the pre-germination treatments, during the first count it was observed that the seeds treated with vegetable regulators showed a higher performance in terms of germination process (74%), also presenting a higher GSI (3.99). These two variables are used to study the seeds vigor. It can be concluded that vegetables regulators contributed to have the seeds expressing their potential in terms of germination and vigor, a condition that was not found when the seeds were only embedded in distilled water (Table 4).

Table 4. Mean of germination percentage in the first count (%G-1C), germination speed index (GSI) and seedling emergence percentage (%E) of the cv. BRS Mel do Cerrado for pre-germination treatments (PGT) with vegetable regulators (VG) and distilled water (DA). Embrapa Cerrados, Planaltina, DF, 2022.

PGT	% G-1C	GSI	%E
RV	74.00 a	3.99 a	94.33 a
DA	00.00 b	0.03 b	93.00 a

Means followed by the same letters in the column do not differ from each other by the F test of the analysis of variance at 1% probability of error.

Table 4 shows that when studying the variable seedlings emergence, no difference in the averages for pre-germination treatments was found. Ferrari et al. (2008) found out that the soaking method did not affect the seeds emergence but decreased the mean emergence time with the use of (GA_{4+7} (250 mg L^{-1}) associated to phenylmethyl aminopurine, similar to the method used in the present study. Zucareli et al. (2009) when using the GA_{4+7}+N-(phenylmethyl)-aminopurine observed an increase in the germination, emergence process and seedlings development of the *P. cincinnata* Mast.. The highest speed of seedling emergence obtained with seeds treated with vegetable regulators, it is probably associated to the action of these substances in stimulating the mobilization of reserves, cell and elongation as mentioned by Taiz e Zeiger (2017).

Oliveira et al. (2020) studied recently harvested seeds of different accesses of *P. alata* and found a percentage of 32.5% for emerged seedlings. Santos et al. (2016), on the other side, analyzed fresh seeds and found 66.25% of emergence while Ferreira et al. (2005) concluded that 59% of the non-stored showed an emergence after the aril removal and soaking the substrate in GA_3 (100 mg L^{-1}). It is possible to note that the emergence percentage observed in the studies mentioned above is lower than the ones found in the present work. The high percentage of seedlings emergence verified in all treatments above 90% (MG = 93,67%) evidenced a higher physiological quality of the cv. BRS MC seeds, which might indicate that the selections performed by the Programa de Melhoramento Genético were also effective when concerning the overcome of obstacles regarding the germination process of the *P. alata* species, although this was not a selection criterion during the selection cycles. Oliveira et al. in 2016 also found out the same when evaluating the seedlings emergence of cv. BRS MC and their genitors.

A fast and uniform seedling emergence is a desirable characteristic for seedlings because the longer the seedling remains in its

initial stages of development, the longer it will be subject to the adverse environment conditions (MARTINS; NAKAGAWA; BOVI, 1999). On the other hand, a slow germination might contribute to an increase in costs to produce a plant and a higher number of seeds is necessary as well as a longer time of the seed in the seedbed to obtain a determined stand of seedlings (GUEDES et al., 2010). Faleiro et al. (2021) report that the cv. BRS MC crop is another type of income for the Brazilian fruit producers, especially small farmers, since it is cheap to produce and shows a good financial return because of its attractive food characteristics and high market value. Besides that, it has a potential to be used as a medicinal and ornamental plant. An adequate seed production logistic and marketing with a guaranteed genetic origin and high physiologic and sanitary quality is necessary so that farmers can have access to the technology allowing the access to a propagation material of excellent quality.

For the technology to reach the producer, it is necessary to have adequate logistics for the production and commercialization of seeds with a guarantee of genetic origin and high physiological and sanitary quality, which will allow access to propagation material of excellent quality.

Conclusion

The maturation stage of fruits associated to the effect of vegetables regulators, GA_{4+7}+N-(phenylmethyl)-aminopurine (300 ppm) showed to influence the final germination percentage although it did not interfere in the seedling emergence of cv. BRS Mel do Cerrado, which was equal or superior to 93% in all treatments. However, seeds treated with vegetable regulators showed a faster seedling emergence.

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