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Scientific Note

Physiological quality of *Physalis ixocarpa* Brot. ex Hornem seeds in relation to maturation stage and growing season[1]

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ABSTRACT

Physalis ixocarpa is an edible fruit with great economic importance in many countries. The use of high-quality seeds is essential for the success of the crop, so their harvest should be carried out at their maximum point of physiological maturity. This study aimed to verify the influence of the growing season and fruit maturation stage on the seed physiological maturity of two *P. ixocarpa* varieties ('green' and 'purple') grown in the Brazilian semi-arid region. The water content, dry weight, germination percentage and seedling emergence were evaluated. The growing season influenced the seed maturation process of the two evaluated varieties, with a more pronounced effect for the 'purple' one. To obtain higher-quality seeds, the cultivation in the Brazilian semi-arid region can be carried out from May to August and the collection of fruits to obtain seeds from the stage 4 of maturation.

KEYWORDS: Solanaceae, tomatillo, fruit maturation, seed physiological potential.

RESUMO

Qualidade fisiológica de sementes de *Physalis ixocarpa* Brot. ex Hornem em relação ao estágio de maturação e época de cultivo

Physalis ixocarpa é uma fruta comestível com grande importância econômica em muitos países. O uso de sementes de alta qualidade é essencial para o sucesso do seu cultivo; portanto, sua colheita deve ser realizada no ponto máximo de maturidade fisiológica. Objetivou-se verificar a influência da época de cultivo e do estádio de maturação dos frutos na maturidade fisiológica de sementes de duas variedades de *P. ixocarpa* ('verde' e 'roxa') cultivadas no semiárido brasileiro. Foram avaliados o teor de água, massa seca, porcentagem de germinação e emergência de plântulas. A época de cultivo influenciou no processo de maturação das sementes das duas variedades avaliadas, com efeito mais pronunciado para a 'roxa'. Para se obter sementes de maior qualidade, o cultivo no semiárido brasileiro pode ser realizado de maio a agosto e a coleta de frutos para obtenção de sementes a partir do estádio 4 de maturação.

PALAVRAS-CHAVE: Solanaceae, tomatillo, maturação de frutos, potencial fisiológico de sementes.

Physalis ixocarpa Brot. ex Hornem, commonly known as "green tomato", "husk tomato" or "tomatillo" (Svobodova & Kuban 2018), belongs to the Solanaceae family. It is traditionally cultivated in Mexico, standing out among the five most important vegetables in the country (González-Pérez & Guerrero-Beltrán 2021, López-Ramos et al. 2021), being used as an ingredient in the preparation of many sauces, due to its thickening capacity and distinctive flavor, both fresh and cooked (Serdar & HanGamze 2018). Besides this, it has an enormous nutritional potential and health benefits, since it is rich in minerals and vitamins, as well as several bioactivities with multiple pharmacological effects, such as antibacterial (Khan et al. 2016), hypoglycemic, antioxidant (Guerrero-Romero et al. 2021) and antitumor activities (Yang et al. 2021).

Since the main form of propagating *P. ixocarpa* is using seeds (García-Osuna et al. 2015), it is essential to use high-quality seeds for a good stand establishment and crop yield. In Brazil, there is little information on the species, so studies are necessary to establish the optimal cultivation conditions for the production of high-quality fruits. However, to obtain seeds with high physiological quality, it is first necessary to determine the ideal harvest time, which normally corresponds to the season when physiological maturity occurs. Developmental and physiological studies are essential to determine seed quality and the suitable harvest time (Gu et al.

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2017, Walia et al. 2018, Kwiatkowski et al. 2020). Environmental conditions during seed development and maturity, including temperature, water stress and excessive rain, nutrient shortage, diseases and pest attack, significantly affect the initial seed quality (Chibarabada et al. 2015, Tanveer et al. 2020, Zhang et al. 2020, Xue et al. 2021).

Despite the existence of studies with *P. ixocarpa* seeds that have examined several aspects, such as the effect of development and drying on the quality and content of sugars (Perez-Camacho et al. 2008), influence of the fatty acid content on longevity (Pichardo-González et al. 2014) and fruit harvest point to obtain seeds with maximum quality (Barroso et al. 2017), to the best of our knowledge, there is little information about how the growing season affects the quality of tomatillo seeds (Tanan et al. 2021), aiming at its commercialization by farmers, as well as germplasm conservation. Thus, this study aimed to verify the influence of the growing season and fruit maturation stage on the seed physiological maturity of two varieties of *P. ixocarpa*, as well as to identify the best time of seed harvest for the production of good quality seeds.

For the production of seedlings of the 'green' and 'purple' *P. ixocarpa* varieties, seeds were obtained from the germplasm collection of the Seed Germination Laboratory of the Universidade Estadual de Feira de Santana (Feira de Santana, Bahia state, Brazil). The seeds were sown in 300-mL plastic cups containing the Vivatto Slim Plus® commercial substrate. After emergence, one seedling per cup was maintained and grown in a greenhouse with 40 % brightness and daily irrigation until completing 20 days. After this period, 120 seedlings were transplanted to field conditions. The seedlings were distributed in rows with spacing of 0.8 m between plants and 2.0 m between rows. Chemical fertilization was carried out with NPK (86.5 kg ha^{-1} of urea, 0.75 kg ha^{-1} of super simple phosphate and 100 kg ha^{-1} of potassium chloride) (Thomé & Osaki 2010). During the cultivation, the branches were staked for support (Muniz et al. 2011). Drip irrigation was applied at 8 a.m. and 4 p.m., during 20 min, with spacing of 0.8 m between the drippers. To repel insects, a solution of emulsified neem oil (1 %; v/v), diluted in water in the proportion of 5 mL L^{-1} (v/v), was applied using a hand sprayer every 15 days (Bado et al. 2005).

The experiments were carried out in two growing seasons, in 2017: May-August (GS1), when there was a higher total rainfall and milder temperatures, and August-November (GS2), when there was a decrease in rainfall and an increase in temperature (Figure 1).

The collection of *P. ixocarpa* fruits of the 'green' and 'purple' varieties was carried out in the GS1 and GS2 growing seasons, when the plants simultaneously had fruits in all maturation stages. In the laboratory, the fruits were grouped into five maturation stages: 1 - green calyx without full expansion and fruit not filling the calyx; 2 - green and fully expanded calyx but fruit not filling the calyx; 3 - green and fully expanded calyx and fruit completely filling the calyx; 4 - green calyx with dry parts, completely expanded and broken, with fruit

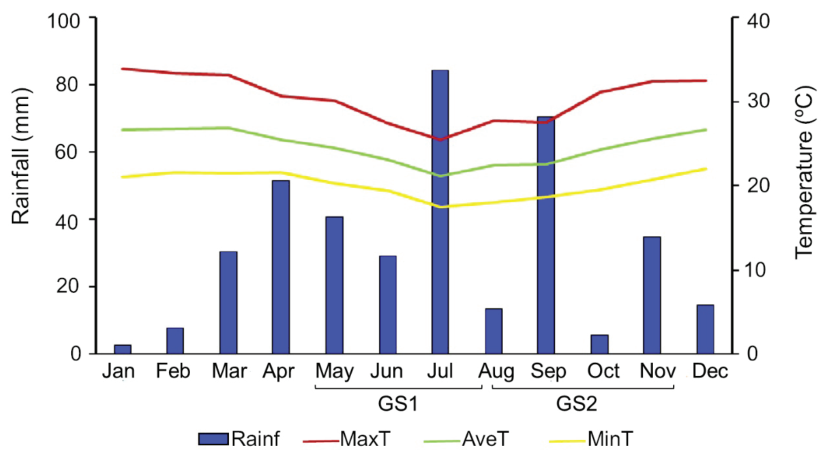

Figure 1. Variations of minimum (MinT), average (AveT) and maximum (MaxT) air temperatures and monthly rainfall (Rainf) recorded in two growing seasons (GS1 and GS2) of *Physalis ixocarpa*, in 2017. Source: Brasil (2018).

completely filling the calyx; 5 - dry calyx, completely expanded and broken, with fruit completely filling the calyx (Figures 2A-J). At each stage, 45 fruits were collected and the seeds were manually extracted from the fruits and washed in running water through a sieve to remove placental tissue and pieces of pulp. After this procedure, the seeds were grouped into five maturity stages: S1 (Figure 2K), S2 (Figure 2L), S3 (Figure 2M), S4 (Figure 2N) and S5 (Figure 2O).

After the seed extraction, 200 seeds of each maturation stage were divided into four replications of 50 seeds each and kept in a forced-air oven at the temperature of 103 ± 2 ºC, during 17 ± 1 h, for determination of water content (wet basis) (ISTA 2019). The difference between initial and final weight was used to calculate the seed water content (%). Together with the water content, the seed dry weight was determined, which consisted of the final average weight of the four subsamples of 50 seeds, with results expressed in mg. The other portion of the seed sample was placed to dry for 72 hours on germitest paper at room temperature (25 ºC ± 1) and relative humidity of 19 ± 3 %. After this procedure, the desiccated seeds were kept in Eppendorf tubes placed in glass flasks at the temperature of 5 ºC ± 1.

In the germination test, 200 desiccated seeds were used for each maturation stage. These seeds were distributed in Petri dishes containing two sheets of germitest paper moistened with a volume of water equivalent to 2.5 times the weight of the paper substrate. The Petri dishes were then kept in a biochemical oxygen demand (BOD) incubator with photoperiod of 12 hours, adjusted in alternating temperatures of 20-30 ºC, for 21 days. Seed germination was evaluated daily, and the seeds were defined as germinated when the radicle was 2 mm. The germination percentage, mean germination time and germination speed index were also estimated.

For vigor analysis, the seed emergence test was used and evaluated daily during 25 days. The seeds of the same aforementioned treatments were sown in styrofoam trays containing the Vivatto Slim Plus® commercial substrate and placed in a controlled environment (40 % of light and daily irrigation). After the end of the emergence, the emergence percentage,

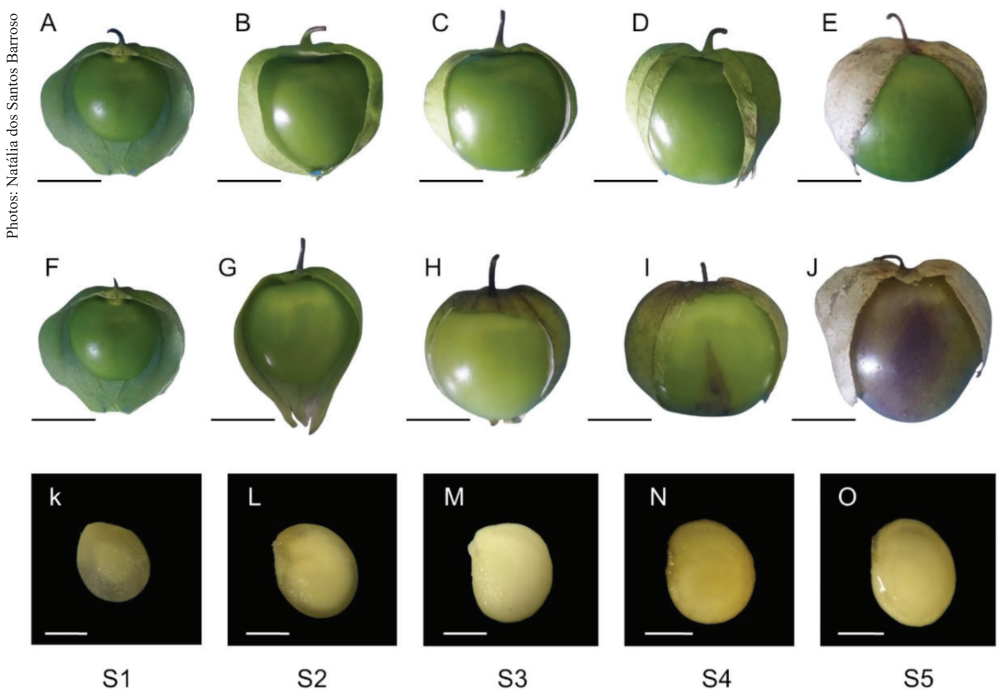

Figure 2. Visual aspect of *Physalis ixocarpa* fruits from the 'green' (A; B; C; D; E) and 'purple' (F; G; H; I; J) varieties and seeds extracted from 'purple' fruits collected at five maturation stages (K to O). Scale bar = 10 mm. S1: green calyx without full expansion and fruit not filling the calyx; S2: green and fully expanded calyx but fruit not filling the calyx; S3: green and fully expanded calyx and fruit completely filling the calyx; S4: green calyx with dry parts, completely expanded and broken, with fruit completely filling the calyx; S5: dry calyx, completely expanded and broken, with fruit completely filling the calyx.

mean emergence time and emergence speed index were calculated (Ranal & Santana 2006). For the seed vigor test, a completely randomized design was used, with 50 seeds per maturation stage and 4 replications, totaling 200 seeds per treatment.

The design used for all assays was completely randomized, in a 2 x 2 x 5 factorial scheme (variety x growing season x maturity stage), with four replications, and each plot was composed of 50 seeds, totaling 200 seeds. Before performing analysis of variance (Anova), the Shapiro-Wilk ($p \leq 0.05$) and Levene ($p \leq 0.01$) tests were applied to detect the normality and homogeneity of variances, respectively. The data on germination and emergence percentages were transformed into arcsine ($\sqrt{x}/100$) (Sorkheh & Amini 2010). The results were then submitted to Anova and the means compared by the F-test or Scott-Knott test at 5 % of probability. All the analyses were performed with the Agricolae package implemented in the R software (R Development Core Team 2021).

The Anova results showed significant effects ($p \leq 0.05$) for the variety, growing season and maturity stages, as well as interactions among the factors in relation to all evaluated traits (Table 1). Considering the triple interaction, there was a significant effect ($p \leq 0.05$) for all the evaluated traits, except for seed water content (Table 1).

The results for water content of the freshly harvested *P. ixocarpa* seeds showed different responses for the studied *P. ixocarpa* varieties, in relation to the growing season and maturity stages (Table 1). The seed water content of the 'green' variety decreased significantly ($p \leq 0.05$) as the seeds were obtained from fruits at more advanced maturation stages. The cultivation period from May to August (GS1), for the seeds extracted from fruits of the 'green' variety in the first two stages of

Table 1. Seed water content, seed dry weight, germination percentage, mean germination time, germination speed index, emergence percentage, mean emergence time and emergence speed index of 'green' and 'purple' *Physalis ixocarpa* seeds collected at different maturation stages and produced in two growing seasons.

Growing season	Maturation stage/*P. ixocarpa* variety									
	S1		S2		S3		S4		S5	
	Green	Purple	Green	Purple	Green	Purple	Green	Purple	Green	Purple
	Seed water content (%)									
GS1	92.8 bC*	79.1 aC	80.9 bC	77.9 bC	67.4 aB	79.4 bC	47.2 aA	54.6 aB	42.2 aA	39.9 aA
GS2	85.1 aB	82.4 aD	67.0 aA	70.7 aC	65.1 aA	71.6 aC	58.9 bA	67.8 bB	54.8 bA	54.1 bA
	Seed dry weight (mg)									
GS1	15.3 bB	41.1 aC	38.4 bC	42.2 bC	67.4 aB	45.9 aC	85.6 aA	63.7 bB	87.1 bA	96.0 aA
GS2	31.0 aE	31.9 bD	42.8 aD	48.7 aC	65.4 aC	47.4 aC	86.5 aB	68.0 aB	96.6 aA	96.0 bA
	Germination (%)									
GS1	-	-	4.0 aC	1.0	6.0 bC	15.0 aB	86.5 aB	91.0 aA	97.5 aA	89.5 aA
GS2	-	-	6.5 aD	-	44.5 aC	4.0 bC	77.5 bB	21.0 bB	96.0 aA	85.5 aA
	Mean germination time (day^{-1})									
GS1	-	-	1.0 aA	0.5	3.7 aB	4.7 aA	4.0 aB	4.9 aB	4.0 aB	4.3 aB
GS2	-	-	5.9 bC	-	5.2 bC	3.9 aB	4.0 aB	7.2 bC	3.5 aB	4.0 aB
	Germination speed index									
GS1	-	-	2.0 aC	0.5	1.4 bC	1.8 aB	11.0 aB	9.5 aB	12.6 bA	10.5 bA
GS2	-	-	0.6 aD	-	5.1 aC	0.4 bC	11.0 aB	1.8 bB	14.9 aA	11.9 aA
	Emergence (%)									
GS1	-	-	4.0	-	7.5 bB	15.5 aA	76.5 aA	81.0 aA	78.5 aA	85.5 aA
GS2	-	-	-	-	26.0 aB	4.0 bC	76.0 aA	17.5 bB	78.5 aA	76.5 bA
	Mean emergence time (day^{-1})									
GS1	-	-	1.0	-	4.6 aB	5.3 aA	5.0 bB	5.1 aA	4.3 bA	4.4 aA
GS2	-	-	-	-	6.1 bC	3.5 bC	4.4 aA	6.2 aB	3.8 aA	5.3 aA
	Emergence speed index									
GS1	-	-	2.0	-	1.4 aB	1.7 aC	8.0 bA	8.2 aB	9.4 bA	9.6 aA
GS2	-	-	-	-	2.3 aB	0.5 bC	9.9 aA	1.5 bB	11.1 aA	7.7 bA

* Means followed by the same lowercase letter (growing season) and uppercase letter (maturation stage) do not differ by the F-test and the Scott-Knott test, respectively, at 5 % of probability. GS1: May-Aug; GS2: Aug-Nov. S1: green calyx without full expansion and fruit not filling the calyx; S2: green and fully expanded calyx but fruit not filling the calyx; S3: green and fully expanded calyx and fruit completely filling the calyx; S4: green calyx with dry parts, completely expanded and broken, with fruit completely filling the calyx; S5: dry calyx, completely expanded and broken, with fruit completely filling the calyx.

physiological maturation, showed high seed water content values (above 80 %), which decreased sharply until reaching 42 %, in the last stage. On the other hand, the seeds from the cultivation carried out from August to November (GS2) showed a reduction from the S2 stage onward, although this did not differ from the subsequent stages. A similar behavior was observed for the 'purple' variety, with a significant reduction ($p \leq 0.05$) of seed water content with advancing maturation stages. The lowest seed water content values [39.9 (GS1) to 54.1 % (GS2)] were obtained from seeds extracted from fully ripe fruits (S5).

Pérez-Camacho et al. (2008) and Barroso et al. (2017) reported seed water content values of 45 and 50 %, respectively, for *P. ixocarpa* seeds extracted from ripe fruits. In the present study, the seeds collected from ripe fruits in the GS1 had water contents of 42 % ('green' variety) and 39 % ('purple' variety), which are quite close to those obtained by Pérez-Camacho et al. (2008). However, the seed water content values measured in the GS2, respectively of 55.0 and 54.1 % for the 'green' and 'purple' varieties, were higher than those found by those authors. This divergence indicates that the seed water content alone may not be a good indicator of physiological maturity, since it may vary according to environmental and genetic influences (Ramos et al. 2021a).

The accumulation of seed dry weight occurred inversely to the seed water content, since there was an increase in this variable as the seeds ripened (Table 1). The maximum seed dry weight values for the 'green' (96.6 mg) and 'purple' (96.0 mg) varieties were obtained when collected from fruits in the S4 and S5 stages, respectively, coinciding with the stages in which greater reductions in water content occurred. These results are consistent with those obtained by Barroso et al. (2017), with *P. ixocarpa* seeds, and by Santiago et al. (2019), with *Physalis angulate* seeds.

The moment of maximum seed dry weight accumulation represents the point of harvesting seeds with maximum physiological quality. This point marks the end of the desiccation and preparation phase for the interruption of trophic connections with the mother plant (Marcos Filhos 2005). However, for some species, the maximum physiological quality occurs before (*Capsicum baccatum* var. *pendulum*; Figueiredo et al. 2017) or after (*P. angulata*; Santiago et al. 2019) the seeds reach the maximum dry weight.

The maximum seed quality can be determined by a combination of attributes, such as germination percentage and vigor. In the present study, a significant effect ($p \leq 0.05$) was observed for both the tomatillo varieties of the maturation stage and growing season interaction, in relation to the germination and seed vigor variables (Table 1).

For the 'green' variety seeds, the germination percentage (G%) and germination speed index (GSI) increased with more advanced maturation stages, reaching the highest values in S5 stage, regardless of the growing season (Table 1). However, seeds of the 'green' variety produced in the GS2 season extracted from fruits in the S3 stage showed the greatest germination percentage (44 %) and GSI (5.1), when compared to the results obtained for the same stage and GS1 (G%: 6; GSI: 1.4), indicating that the environmental conditions in the second maturation stage accelerated the acquisition of germination capacity. A similar behavior was observed for germination percentage and GSI of 'purple' seeds, with the highest averages of these traits found in the fifth maturation stage in both the growing seasons. However, the seed germination potential for this variety was reached sooner in the GS1, since the highest values of germination percentage and GSI were reached respectively from seeds collected in the third (15 %; 1.8) and fourth (91 %; 9.4) stages.

Likewise, other authors also observed an increase in the germination percentage and GSI with the advance of *P. ixocarpa* seed maturation, with the highest averages for these variables being obtained from fruits in the last two maturation stages (Pérez-Camacho et al. 2008, Barroso et al. 2017). The influence of the environment on seed germination in the present study corroborates the results reported by Diniz & Novembre (2019), who found an environmental effect during seed formation on germination, GSI and mean germination time, as well as on the seed maturity timing of *Physalis peruviana*.

In relation to the mean germination time, a similar behavior was observed between the studied *P. ixocarpa* varieties (Table 1). Seeds from fruits harvested in the early development stages (S1 and S2) had the lowest mean germination time, ranging from 0 to 5.9 days for the 'green' and from 0 to 4 days for the 'purple' variety, during the two evaluated periods. The mean germination time is the weighted average of the germination time of seeds in the sample (Soltani et al. 2015).

Based on the vigor of 'green' and 'purple' *P. ixocarpa* seeds, the emergence percentage, emergence speed index and emergence time were significantly influenced by the association of the growing season and maturation stages (Table 1). Similarly to the germination parameters, there was an increase in the emergence percentage and emergence speed index, and a decrease in the mean emergence time, during the seed development. For the 'green' and 'purple' varieties, there was an increase in the emergence percentage and emergence speed index and a decrease in the mean emergence time for the GS2 and GS1, respectively. These results indicate that the environmental conditions and the interaction of the *P. ixocarpa* varieties with the environment during the formation and maturation of the seeds influenced the germination capacity and seed vigor.

It was observed that the association among the analyzed parameters for the 'green' and 'purple' *P. ixocarpa* was verified in the fifth stage in both the growing seasons (Figures 3A-D). This stage is indicated for the retrieval of seeds with better physiological quality and, at this stage, there was a higher germination percentage, and the best seed vigor coincided with the lowest water content and highest dry weight (Figure 3). This is probably related to the increase in the content of total soluble sugars in the final stages of development, which acts to stabilize the membrane of organelles, increasing the tolerance to desiccation and improving the performance of the embryo during germination and emergence (Marcos Filho 2005, Perez-Camacho et al. 2008). Similarly, Ramos et al. (2021b) also reported that a maximum physiological potential of *P. angulata* is acquired when seeds show a high vigor and germination, what coincides with the maximum accumulation of dry

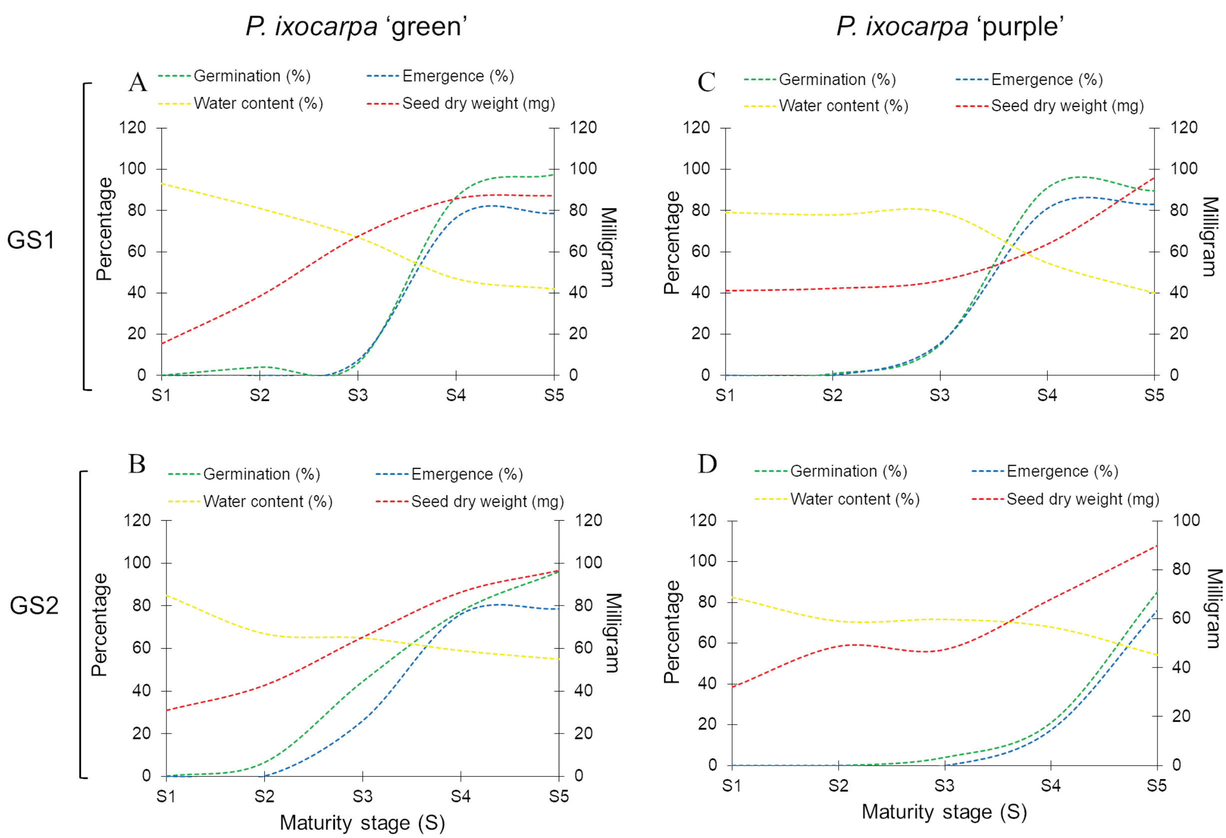

Figure 3. Relationship among the traits water content, dry weight, germination and seedling emergence of dry seeds of 'green' (A-B) and 'purple' (C-D) *Physalis ixocarpa* extracted from fruits collected at five maturation stages and cultivated in two growing seasons. GS1: May-Aug; GS2: Aug-Nov. S1: green calyx without full expansion and fruit not filling the calyx; S2: green and fully expanded calyx but fruit not filling the calyx; S3: green and fully expanded calyx and the fruit completely filling the calyx; S4: green calyx with dry parts, completely expanded and broken, with fruit completely filling the calyx; S5: dry calyx, completely expanded and broken, with fruit completely filling the calyx.

weight, when seeds reach physiological maturity. Future research can focus on more genotypes in different experimentation areas and develop plans to identify the potential areas and optimum agronomic practices for tomatillo cultivation in Brazil.

For the conditions analyzed in this study, the growing season influenced the maturity of the 'green' and 'purple' *P. ixocarpa* seeds. In order to obtain higher quality seeds, the cultivation of these varieties can be carried out from May to August in the conditions of the Brazilian semi-arid, and the collection of fruits to obtain seeds can be carried out from the fourth maturity stage (green calyx with dry parts, completely expanded and broken, with fruit completely filling the calyx).

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