AGRONOMY (AGRONOMIA)

Revista Brasileira de Ciências Agrárias
ISSN (on line) 1981-0997
v.13, n.4, e5574, 2018
Recife, PE, UFRPE. www.agraria.pro.br
DOI:10.5039/agraria.v13i4a5574
Protocolo 5574 - 15/03/2018 • Aprovado em 28/06/2018

Physiological maturity of *Solanum sisymbriifolium* seeds

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ABSTRACT: The objective of this work was to evaluate the physical and physiological quality of juá seeds (*Solanum sisymbriifolium* Lam.), in five maturation stages, treated with three concentrations of GA3 and submitted to the cold test. The biometric characteristics of the fruits, thousand mass and water content of the seeds, standard test and mean germination time, percentage and mean time of normal seedling formation were determined. The fruit biometry was analyzed using estimated parameters using descriptive statistics. For the other variables, a completely randomized experimental design was used in a 5 x 2 x 3 triple factorial scheme, with five maturation stages: stage 1 (green fruits), stage (2 light green fruits), stage 3 (yellow fruits), stage 4 orange fruits), and stage 5 (red-carmine fruits); seeds submitted or not to the cold test and; three concentrations of gibberellic acid (GA3), 0% (water), 0.025% (250 mg L^{-1}) and 0.050% (500 mg L^{-1}), with four replicates of 25 seeds each treatment. The analysis of variance showed interaction between the maturation stage of the fruits and the doses of GA3, where the maturation stages 3, 4 and 5, together with the solutions of GA3, favored the germination of the seeds. The formation of normal seedlings was higher from seeds obtained from fruits at the maturation stage 5.

Key words: gibberellic acid; juá; overcoming dormancy; physiological quality; vigor

Maturidade fisiológica de sementes de *Solanum sisymbriifolium*

RESUMO: O objetivo neste trabalho foi avaliar a qualidade física e fisiológica de sementes de juá (*Solanum sisymbriifolium* Lam.), em cinco estádios de maturação, tratadas com três concentrações de GA3 e submetidas ao teste de frio. Foram determinadas as características biométricas dos frutos, a massa de mil e o teor de água das sementes, assim como os testes de: porcentagem de sementes germinadas e tempo médio de germinação, germinação (formação de plântulas normais), porcentagem e tempo médio de formação de plântulas normais. A biometria dos frutos foi analisada por meio de parâmetros estimados utilizando estatística descritiva. Para as demais variáveis foi utilizado delineamento experimental inteiramente casualizado em esquema fatorial triplo 5 x 2 x 3, sendo cinco estádios de maturação dos frutos: estádio 1 (frutos verdes), estádio (2 frutos verde-claros), estádio 3 (frutos amarelos), estádio 4 (frutos alaranjados) e, estádio 5 (frutos vermelho-carmim); sementes submetidas ou não ao teste de frio e; três concentrações de ácido giberélico (GA3), 0% (água), 0,025% (250 mg L^{-1}) e 0,050% (500 mg L^{-1}), com quatro repetições de 25 sementes cada tratamento. A análise de variância mostrou interação entre o estádio de maturação dos frutos e as doses de GA3, em que, os estádios de maturação 3, 4 e 5, em conjunto com as soluções de GA3, favoreceram a germinação das sementes. A formação de plântulas normais foi maior a partir de sementes obtidas de frutos no estádio de maturação 5.

Palavras-chave: ácido giberélico; juá; superação de dormência; qualidade fisiológica; vigor

Introduction

Solanum sisymbriifolium Lam. (Solanaceae) is an herbaceous or subshrub species, widely distributed in Brazil and also in the temperate and tropical regions of South America. It inhabits preferentially areas of cerrado, caatinga, rupestrian field and altitude field, rocky outcrops, forest edge, impacted field and pastures, at altitudes that can reach 2120 m (Smith & Downs, 1966).

The species is known in Brazil as *juá, juá-das-queimadas, juá-meso, joá, joá-de-capote, joá-bravo, arrebenta-cavalo* and *mata-cavalo* (Moreira & Bragança, 2011) for being overlaid by long, rigid, strong, straight and rust-yellow spines on the branches, petioles, ribs, peduncles, shafts and calyxes. The fruit is globose and yellow-reddish when ripe (Kissmann & Groth, 2000).

The plant is as an invasive herb in grazing areas, crops and road borders (Srivastava et al., 2015). It has been considered resistant to cyst nematodes, therefore, it has been used as a trap crop to reduce nematode infestation levels in the soil (Pestana et al., 2009; Conceição et al., 2012). The fruit are a source of solasodine, a substance used in the synthesis of corticosteroids (anti-inflammatory) and sex hormones (Hill & Hulley, 1995). Its root decoction is used as a resolving and unclogging of digestive and urinary organs, such as diuretic, analgesic, contraceptive, antiseptic and hepatoprotective, while aerial parts are used in the treatment of diarrhea and respiratory and urinary tract infections (Mentz et al., 1997).

The species *S. sisymbriifolium* propagates by means of seeds (Moreira & Bragança, 2011); however, the plant's harvesting point is not found in the literature. According to Aguiar et al. (1988), the physiological maturation of the seeds is usually accompanied by changes in the external appearance, such as the coloring of the fruit and the seeds. These characteristics are important for guidance about the ideal harvesting point for seeds (Aguiar et al., 2007). However, according to Carvalho & Nakagawa (2012), the lack of uniformity in the development and coloring of fruit and seeds in the same plant makes it difficult to determine the physiological maturity time of the seeds, as in *S. sisymbriifolium.*

Another problem found in seeds of *S. sisymbriifolium* is that they have primary dormancy, which, according to Marcos Filho (2015), is developed during maturation. In general, after physiological maturity, the seeds go through a latency period, in which adequate conditions of the environment allow them to start the process of imbibition and to continue germination. However, seeds of some species, even in favorable conditions of the environment, present problems in the germination and this can occur due to lack of certain hormones, needing specific treatments. These seeds are called dormant seeds (Montório et al., 1997).

In many species, the nature of dormancy is physiological, caused by the regulation between hormones. In this case, abscisic acid (ABA) inhibits germination, whereas gibberellic acid (GA_3) promotes germination; the antagonism between ABA and GA_3 plays a key role in the control of seed germination (White et al., 2000).

In some species with physiological dormancy caused by deficiency in GA_3, specific treatments with exogenous hormones, such as GA_3, promote germination (Baskin & Baskin, 2004). The gibberellins have a very important role in the germination process of the seeds also by unifying germination and acting mainly in the cellular stretching, concomitant with the emission of the radicle (Braun et al., 2010).

The objective of this work was to evaluate the physical and physiological quality of *S. sisymbriifolium* seeds, obtained from fruit at different stages of maturation based on exocarp staining, treated with different concentrations of GA_3.

Material and Methods

The fruit of S. *sisymbriifolium* were collected directly in the plants, found in an urban area of the city of Cuiabá/MT. After the fruit were collected, they were taken to the Seeds Laboratory of the Federal University of Mato Grosso - UFMT, where a visual evaluation was carried out to separate the lots according to maturation stages, based on the exocarp staining through the color table elaborated by Kaiser et al. (2016). The fruit of *juá* were separated (Figure 1) in five maturation stages: green fruit (stage 1), light green fruit (stage 2), yellow fruit (stage 3), orange fruit (stage 4) and carmine red fruit (stage 5).

Then, the biometry was evaluated by measuring 30 fruit, randomly taken from each stage of maturation. The length and diameter of the fruits were obtained with a digital caliper (0.01 mm). The length of the fruit, without the peduncle, was measured from the base to the apex, and the diameter at the midline. The fresh matter mass of the fruit was determined by analytical weighing (0.0001 g). It was also determined the number of seeds per fruit.

The seeds were manually extracted using a scalpel to open the fruits and placed on paper towels on a laboratory bench for 24 hours. After this period, the mass of one thousand seeds was determined using eight subsamples

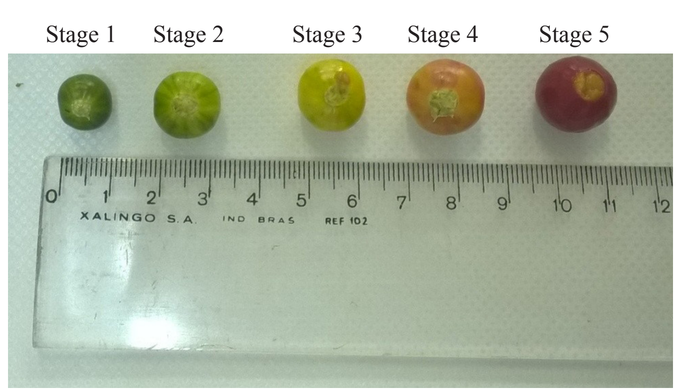

Figure 1. Maturation stages of fruit of (*Solanum sisymbriifolium*) according to exocarp staining. Stage 1 (green fruit), stage 2 (light-green fruit), stage 3 (yellow fruit), stage 4 (orange fruit) and stage 5 (carmine red fruit).

of 100 seeds and the water content was determined by the greenhouse method at 105 ± 3º C for 24 hours, for each stage of maturation (Brazil, 2009). Afterwards, the germination test was carried out in a completely randomized experimental design in a 5x2x3 triple factorial scheme, with five stages of maturation of the seeds, submitted or not to cold test and gibberellic acid (GA_3) at concentrations of 0% (water), 0.025% (250 mg L^{-1}) and 0.05% (500 mg L^{-1}), with four replicates of 25 seeds for each treatment.

For the cold test, seeds of each maturation stage, after being sown on two sheets of blotting paper moistened with the respective gibberellic acid solutions, were kept for seven days in a BOD incubator at a temperature of 10° C, in the dark. After this period, the seeds were relocated in a BOD incubator, regulated at the alternating temperature of 20/30° C, with a 12-hour photoperiod.

The seeds were seeded in transparent gerbox boxes (11 x 11 x 3 cm) on two sheets of blotting paper moistened with the respective solutions of gibberellic acid until saturation. After sowing, the boxes were wrapped with plastic film, capped and kept in a BOD-type germination incubator under alternating temperature 20/30° C, with a 12-hour photoperiod, according to recommendations of RAS (Brazil, 2009) for species of the genus *Solanum*.

The percentage of germinated and germinating (normal seedling formation) seeds was determined and, for the calculation of the average germination time (AGT) and the average seedling formation time (ASFT), we used the equation proposed by Maguire (1962). Germinated seeds and normal seedling formation were daily evaluated until the 20th day after sowing, when normal seedling formation stabilized. The seeds were considered germinated when the protrusion of the primary root with 2 mm length occurred and the normal seedlings were considered as those that presented the structures of aerial part and root developed.

The results were submitted to analysis of variance and the comparison of averages was made by the Tukey test, at the level of 1% of probability, using the statistical program Assistat (Silva & Azevedo, 2016).

Results and Discussion

Table 1 presents the data of the biometrics of *S. sisymbriifolium* fruit at the five maturation stages. The highest averages of length, diameter, fresh mass and number of seeds per fruit were obtained at stages 3, 4 and 5 of maturation. According to Abud et al. (2013), the variation in the biometric estimates can occur due to the physiological changes that provoke increases in the values of these variables throughout the maturation of the fruit.

The length and diameter of the fruit, both at maturation stage 1 and 2, presented negative asymmetric coefficients, which represents, within these maturation stages, predominance of fruit with values of length and diameter closer to the maximum value. The same pattern was observed for fresh mass and number of seeds per fruit at maturation stages 2 and 1, respectively, with asymmetric negative coefficients. For the other maturation stages, the asymmetry coefficients were positive, indicating that within each stage of maturation there was predominance of fruit with values of biometric variables evaluated closer to the minimum (Table 1). All the biometric variables presented a platicuric distribution, according to the kurtosis coefficient (K<3). This indicates that the frequency distribution of the variables analyzed presents a larger amplitude of the data (Table 1).

Table 1. Biometric parameters of *juá* (*Solanum sisymbriifolium*), according to fruit maturation stages.

Characteristics	Stages	Min.	Max.	X	SD	CV	S	K
Length (mm)	1	7.12	11.58	9.29 c	5.10	14.65	-0.25	-0.32
	2	7.08	12.25	9.64 c	5.53	15.30	-0.01	-0.82
	3	9.70	13.34	11.06 b	3.55	8.55	0.46	0.05
	4	9.60	13.37	11.39 b	3.99	9.33	0.45	-0.69
	5	11.13	14.49	12.55 a	3.05	6.48	0.41	0.52
Diameter (mm)	1	7.61	13.21	10.41 c	5.99	15.34	-0.41	-0.61
	2	7.34	13.79	10.81 c	7.32	18.05	-0.25	-1.17
	3	10.56	14.14	12.12 b	3.35	7.36	0.50	0.46
	4	10.53	14.77	12.57 b	3.78	8.03	0.10	-0.19
	5	12.03	16.43	13.62 a	4.00	7.84	0.72	0.59
Massa (g)	1	0.27	1.20	0.67 c	1.00	40.18	0.05	-0.79
	2	0.27	1.31	0.76 c	1.29	45.32	-0.08	-1.33
	3	0.77	1.64	1.12 b	0.91	21.74	0.89	0.21
	4	0.79	1.71	1.16 b	0.93	21.35	0.70	-0.27
	5	1.11	2.53	1.65 a	1.16	18.73	0.99	1.64
Number of seeds	1	28	164	99.2 b	128.67	34.59	-0.08	-0.51
	2	35	150	91.2 ab	119.49	34.93	0.15	-0.54
	3	61	159	103.3 ab	95.29	24.59	0.28	-0.04
	4	65	146	99.6 ab	77.23	20.68	0.54	0.37
	5	73	157	111.9 a	77.19	18.40	0.07	0.35

Averages followed by the same lowercase letter in the column do not differ statistically from each other by the Tukey test at the 1% probability level. Min = minimum value, Max = maximum value, X = average, SD = standard deviation, CV = Coefficient of Variation, S = asymmetry, and K = kurtosis. Stage 1 - green fruit, stage 2 – light-green fruit, stage 3 - yellow fruit, stage 4 - orange fruit and stage 5 – carmine red fruit.

With advancement at the stage of maturation of the fruit, a decrease in the water content of the seeds was observed. The result was inversely related to the mass of one thousand seeds that increased according to the progress of maturation (Table 2). Marcos Filho (2015) points out that, as the seeds mature physiologically, they accumulate dry matter, consequently the mass of a thousand seeds increases, and simultaneously there is a natural reduction in the water content of the seeds. All this process is directly related to the increase of the physiological potential of the seeds, considering that the vigor increases with the maturation, reaching the maximum value in the near or coinciding moment with the maximum accumulation of reserves.

In a study with melon (*Cucumis melo* L.) seeds, Donato et al. (2015) reported that those from immature fruit did not reach maximum development, resulting in low-mass seeds due to the lower accumulated reserves. Mendonça et al. (2008), studying tomato seeds harvested at different stages of fruit maturation, found that immature fruit provide lower dry matter content on the seeds and that the lower the maturation stage of the fruit, the greater the unevenness of the seeds inside the them.

The analysis of variance (Table 3) shows that the cold test did not influence the germination and normal seedling percentages nor the mean time of seedling formation. However, there was interaction between the stage of maturation of fruit and the doses of GA_3, with significant effect of the doses of the plant regulator applied to overcome the dormancy of *S. sisymbriifolium* seeds. Thus, the percentage data of germinated seed and germination (normal seedlings), the average germination and seedling formation time were discussed based on the interaction between the factors maturation of fruit and doses of GA_3.

The percentage of germinated seeds from seeds extracted from fruit at maturation stage 1 was low, even with the application of GA_3 (Table 4). This fact may be related to the incomplete development of the embryo because, possibly, these seeds did not reach the point of physiological maturity. According to Figueiredo et al. (2017), at this stage of development not all seeds have a fully formed embryo, thus being immature to germinate. In addition to this, there is a possibility of seed dormancy at this initial stage, which directly affects the actual germination potential (Justino et al., 2015). Queiroz et al. (2011) state that the occurrence of dormancy associated with the physiological immaturity of the seeds justifies reduced germination and vigor.

Table 3. Summary of the analysis of variance of the data referring to the percentage of germinated seeds (G), percentage of normal seedlings (NS), average germination time (AGT) e average normal seedling formation time (ASFT) of *juá* (*Solanum sisymbriifolium*).

Cause of variation	DF	F values			
		G	NS	AGT	ASFT
		%		Days	
Maturation (M)	4	135.98**	37.70**	4.48**	11.72**
Cold Test	1	3.66^{ns}	0.23^{ns}	19.95**	1.49^{ns}
Giberellic acid (GA_3)	2	252.61**	97.19**	44.16**	4.09*
Interaction ($MxGA_3$)	8	10.94**	4.19**	4.99**	4.23**
CV (%)		18.61	35.03	24.22	21.62

**, *, ^{ns} Significant at the level of 1 and 5% probability and not significant, respectively. DF = degree of freedom; CV = coefficient of variation.

Table 4. Percentage of germinated seeds and formation of normal seedlings of *juá* (*Solanum sisymbriifolium*) according to fruit maturation stages and gibberellic acid (GA_3) concentrations.

Maturation	Water	GA_3 (%)	
		0.025	0.05
Germinated seeds (%)			
1. Green	6 bB	23 cA	10 cB
2. Light-green	26 aB	72 bA	68 bA
3. Yellow	30 aB	84 abA	84 aA
4. Orange	36 aB	85 abA	82 abA
5. Carmine red	29 aB	92 aA	93 aA
CV (%)		18.61	
Normal seedlings (%)			
1. Green	2 bB	18 cA	9 cAB
2. Light-green	15 abB	56 abA	52 abA
3. Yellow	12 abB	52 bA	49 bA
4. Orange	24 aB	51 bA	61 abA
5. Carmine red	17 abB	71 aA	69 aA
CV (%)		35.03	

Averages followed by the same lowercase letter in the column do not differ statistically from each other by the Tukey test at the 1% probability level.

The increase of seed maturation, together with the application of GA_3, influenced the percentage of germinated seeds and the formation of normal seedlings (Table 4), and maturation stages 2, 3, 4 and 5 contributed to higher percentages of germinated seeds, with a positive effect of GA_3, regardless of the applied concentration. Queiroz et al. (2011) explain that when the seeds reach physiological maturity, they are able to germinate and generate normal seedlings, presenting all the necessary chemical and physiological apparatus. Ricci et al. (2013) also observed a direct relationship

Table 2. Water content (%) and mass of one thousand seeds (g) of *juá* (*Solanum sisymbriifolium*), according to fruit maturation stages and gibberellic acid (GA_3) concentrations the maturation stages of the fruit.

Maturation	Water content			Mass of one thousand seeds(g)		
	%	SD	CV (%)	g	SD	CV (%)
1. Green	9.5	0.143	1.5	1.6	0.009	5.4
2. Light-green	9.3	0.230	2.5	1.2	0.008	6.9
3. Yellow	8.9	0.279	3.1	1.9	0.008	4.3
4. Orange	8.6	0.613	7.2	2.1	0.007	3.2
5. Carmine red	8.9	0.269	3.0	2.1	0.009	4.4

SD = Standard Deviation; CV= coefficient of variation.

between the fruit maturation and the germination percentage of the *jalapeño* pepper (*Capsicum annuum*) seeds. The higher vigor and physiological quality of seeds harvested from fruit with older age explain this relationship (Nakada et al., 2011).

In relation to the positive effect of the use of gibberellin for seed dormancy, Braun et al. (2010) report that the plant regulator GA_3 is directly involved in the seed germination process and improves seedling performance, accelerating seedling formation velocity and enhancing seed potential. The gibberellins present a stimulatory effect on the germination process when applied to dormant and also non-dormant seeds (Almeida et al., 2015).

For normal seedling percentage, the best result was obtained at the maturation stage 5, regardless of GA_3 concentration (Table 4). It was also observed by Gonçalves et al. (2015) a greater vigor and physiological quality of the seeds of bonnet pepper (*Capsicum chinense* Jacq.) harvested from fruit with more advanced stage of maturation. According to Carvalho & Nakagawa (2012), seeds that are not completely mature can germinate, however, they do not result in seedlings as vigorous as those harvested at the appropriate time. In general, seeds acquire the highest quality close to physiological maturity, during which time the maximum accumulation of dry mass occurs, promoting the complete formation of biochemical, morphological and structural systems (Nakada et al., 2011).

The fruit maturation stage did not influence the average seed germination and normal seedling formation times, with the exception of those sown on paper moistened with distilled water, without addition of GA_3. In these cases, the average germination time and the average seedling formation time was lower at the maturation stage 1, which are the greener seeds (Table 5). However, it is necessary to consider that the percentage of germinated seeds at this maturation stage was lower (Table 4).

Table 5. Average germination time (AGT) and average normal seedling formation time (ASFT) of *juá* (*Solanum sisymbriifolium*) according to fruit maturation stages and gibberellic acid (GA_3) concentrations.

Maturation	Water	GA_3 (%)	
		0.025	0.05
TMG			
1. Green	8.1 aA	11.0 aA	8.0 Aa
2. Light-green	15.1 bB	11.0 aA	9.0 Aa
3. Yellow	17.0 bB	8.8 aA	9.0 aA
4. Orange	15.0 bB	10.3 aA	9.0 Aa
5. Carmine red	15.0 bB	8.5 aA	8.5 aA
CV (%)		24.22	
TMFP			
1. Green	5.6 bB	14.2 aA	14.5 aA
2. Light-green	17.5 aA	16.7 aA	16.7 aA
3. Yellow	13.1 aA	15.9 aA	16.1 aA
4. Orange	17.1 aA	16.5 aA	16.5 aA
5. Carmine red	17.3 aA	16.1 aA	16.5 aA
CV (%)		21.62	

Averages followed by the same lowercase letter in the column do not differ statistically from each other by the Tukey test at the 1% probability level.

The use of GA_3 reduced the average germination time of the seeds obtained from the fruit in the maturation stages 2 to 5. The seedling germination and formation speed is an important factor, because the faster the growth, the lower the susceptibility of seedlings to bad weather.

The seeds of *S. sisymbriifolium* present primary dormancy, that is, dormancy is established during seed development, because even when subjected to favorable conditions of substrate, water, temperature, oxygen and light, they have difficulty germinating. However, by using GA_3, germination was stimulated, reaching more than 90%, considerably higher than the highest percentage of germination obtained without the use of GA_3, which was 36%. According to Silva et al. (2013), the use of GA_3 aims to overcome, basically, the physiological dormancy of seeds.

Conclusions

The fruit maturation stage influences the physiological quality of *S. sisymbriifolium* seeds, with yellow (stage 3), orange (stage 4) and carmine red (stage 5) colors being the most suitable for seed production, using 0.025% gibberellic acid solution (GA_3).

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