X-RAY AND IMAGE ANALYSES OF SEEDS OF *Passiflora cincinnata* AND *Passiflora morifolia*

TESTES DE RAIOS-X E ANÁLISE DE IMAGENS DE SEMENTES DE *Passiflora cincinnata* E *Passiflora morifolia*

Júlia Janice Loffler[1]
https://orcid.org/0000-0001-6104-0362
Beatriz Fernanda Silva Lima[2]
https://orcid.org/0000-0003-2983-6963
Severino de Paiva Sobrinho[3]
https://orcid.org/0000-0002-7989-8145
Petterson Baptista da Luz[4]
https://orcid.org/0000-0003-4067-0087

Submetido: 07/12/2022 / Aprovado: 19/01/2023 / Publicado: 11/05/2023

Abstract

Wild species of passion fruit present characteristics such as resistance to diseases and pests, longevity, and adaptability to adverse climatic conditions. Morphological analyses of seeds of wild species are frequently performed to investigate reproductive aspects of plants used in genetic improvement programs. The present study evaluated the quality of seeds of *Passiflora cincinnata* and *Passiflora morifolia* at different stages of fruit development, through X-ray and GroundEye® software image analyses. Four repetitions of 50 seeds and four stages of fruit maturation were used. X-ray-exposed seeds were classified as "full" or "empty". The computerized image analysis evaluated 10 descriptors related to color dominance and 7 descriptors related to geometry. A germination test was subsequently performed. In X-rayed samples, a higher percentage of empty seeds was detected in the green phase of maturation (6% for *P. cincinnata* and 11% for *P. morifolia*). GroundEye software® findings indicated that differences in coloration may indicate differences in the physiological quality of seeds. The collective results indicate that fruits of *P. cincinnata* and *P. morifolia* can be collected at the "on time", mature, and senescent maturation stages without compromising the physiological quality.
Keywords: Groundeye®. Passion fruit. Vigor. Germination.

Resumo

Espécies silvestres de maracujá têm apresentado características como resistência a doenças e pragas, longevidade e adaptabilidade às condições climáticas adversas. Análises morfológicas de sementes de espécies silvestres são frequentemente adotadas, visando investigar aspectos

[1]Mestre. Mato Grosso State University - Graduate Program in Genetics and Plant Breeding, Brazil. E-mail: juliajaniceloffler92@gmail.com
[2]Mestre. Mato Grosso State University - Graduate Program in Genetics and Plant Breeding, Brazil. E-mail: beatrizfernanda_lima@hotmail.com
[3]Doutor. Mato Grosso State University - Cáceres Campus, Brazil. E-mail: paivasevero@unemat.br
[4]Doutor. Mato Grosso State University - Cáceres Campus, Brazil. E-mail: petterson@unemat.br

DOI: http://dx.doi.org/10.24021/raac.v20i1.7443 V. 20, N. 1 (2023)

reprodutivos de plantas a serem aplicadas em programas de melhoramento genético. Considerando esses pontos, o presente estudo tem como objetivo avaliar a qualidade das sementes de *Passiflora cincinnata* e *Passiflora morifolia* em diferentes estágios de desenvolvimento do fruto, por meio de testes de raios-X e análise de imagens, utilizando o software GroundEye®. Para a execução dos testes, foram utilizadas quatro repetições de 50 sementes e quatro estádios de maturação dos frutos. As sementes submetidas à exposição radiográfica foram classificadas como "cheias" ou "vazias". Na análise computadorizada de imagens, foram avaliados dez descritores relacionados à dominância de cores e sete descritores relacionados à geometria e posteriormente realizados o teste de germinação. Os testes radiográficos apresentaram maior porcentagem de sementes vazias, 6% para *P. cincinnata* e 11% para *P. morifolia*, na fase verde de maturação. Os resultados obtidos pelo software GroundEye® indicaram que as diferenças de coloração podem indicar diferenças de qualidade fisiológica de sementes. Os frutos de *P. cincinnata* e *P. morifolia* podem ser coletadas nos estádios de maturação "de vez", maduro e senescentes, sem comprometer a qualidade fisiológica.
Palavras-chave: Groundeye®. Maracujá. Vigor. Germinação.

1. INTRODUCTION

Uncultivated passion fruit species have been presenting characteristics as resistance to diseases and pests, longevity, adaptability to adverse weather conditions and other not explored potentialities. Among these species, *P. nitida*, *P. caerulea*, *P. laurifolia*, *P. suberosa*, *P. alata*, *P. coccinea*, *P. gibertii*, and *P. setácea* are highlighted. According to Battilani *et al.* (2012), morphologic analyses of seeds of wild species are frequently adopted, aiming to investigate plant reproductive aspects to be applied in genetic improvement programs, as well as to assist studies about germination process, resistance to diseases and higher productivity and fruit quality.

Automated methods allow the categorization of seeds through images, being efficient to permit the identification of seeds of various colors, the presence of mechanical damages, and the classification of different sizes. Literature has documented many studies about image analysis, in order to establish a pattern of vigor in seeds (KOBORI *et al*., 2012; FARIA *et al.*, 2021).

Among the methods to evaluate the intern morphology of the seeds, X-ray analysis is highlighted. The X-ray tests offered a quick and efficient evaluation of seed intern structures in most of the forest and agricultural species, beyond it is not a destructive method. The development of radiography methodology seeks the improvement of seed allotment selection with better quality, carried out by the disposal of seeds with internal damage, beyond it is an important seed bank technique to ex situ conservation of germplasm, guaranteeing the storage of those with higher viability, providing information about morphologic anomalies or eventual damages that can harm the germination (KOBORI *et al.*, 2012).

Another technique that can be used on seed morphology evaluation is the use of computerized analysis, which intends, through image capture of the seeds, the categorization of aggregated characteristics to size, color, format, texture, among others, acquired fast. However, studies related to seed computerized analysis are incipient, which need pertinent researches to that aspect, especially in what refers to the association of methodologies to evaluate the seed quality by morphologic characteristics (PINTO *et al.*, 2015). In this way, image analysis techniques can help in choosing the correct physiological maturity point of *Passiflora* seeds.

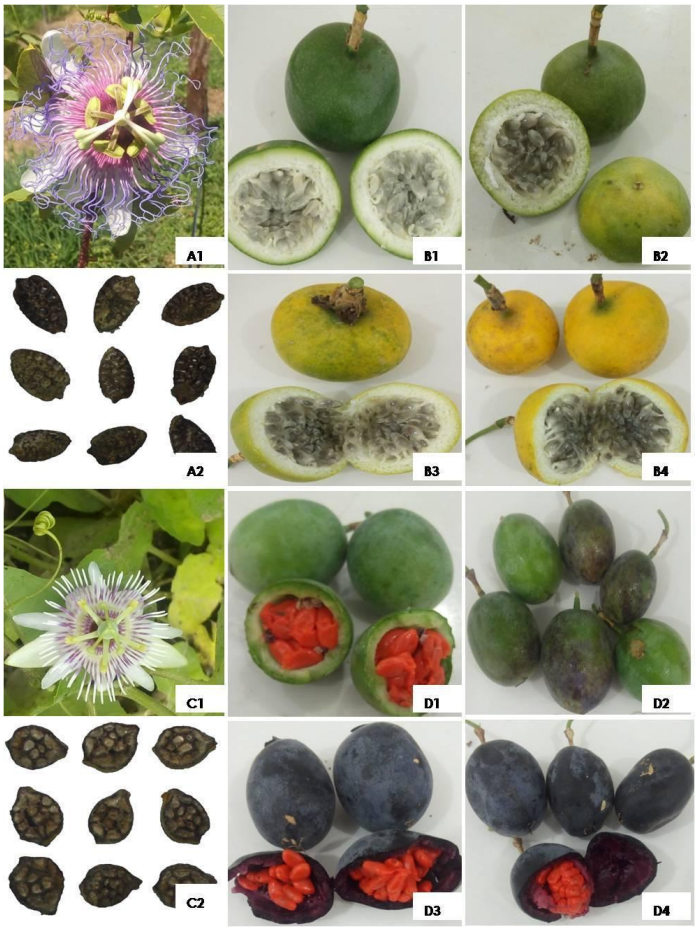
DOI: http://dx.doi.org/10.24021/raac.v20i1.7443 V. 20, N. 1 (2023)

Considering the facts, the present study aims to evaluate the quality *P. cincinnata*.and *P. morifolia* seeds in the function of the fruit development stage through X-rays tests and image analysis, by GroundEye® system.

2. MATERIAL AND METHODS

P. cincinnata e *P. morifolia* species were studied in four maturation stages of the fruit, presented in figure 1. The seeds used on the experiments were obtained at the Active Germplasm Bank, implanted in the experimental field, in an area adjacent to the University Campus of Caceres. The region where the seeds were collected has tropical weather (Aw), dry winter, rainy summer, and an average temperature around 26º C, which can reach 41º C. Annual rainfall is approximately 1.335 mm, mainly concentrated in the months of December to March (NEVES *et al.*, 2011).

Figure 1. A1 e A2 – Flower and seed of *Passiflora cincinnata*, respectively. B1, B2, B3, B4 – Maturation stages of *Passiflora cincinnata* fruit: green, breaker, ripe, and overripe, respectively. C1 and C2 – Flower and seed of *Passiflora morifolia*, respectively. D1, D2, D3, D4 – Maturations stages of *Passiflora morifolia*: green, breaker, ripe, and overripe, respectively.



The seeds were obtained from fruits in four maturation stages: green, breaker, ripe, and overripe (Table 1). After collection, the fruits were submitted to an extraction process and cleaning of the seeds. Pulp and seeds were rubbed on a sieve with hydrated lime and posteriorly washed with running water until the mucilage was completely removed. After the cleaning process, the

DOI: http://dx.doi.org/10.24021/raac.v20i1.7443 V. 20, N. 1 (2023)

seeds rest in an absorbent paper at the stand to dry, for 24 hours, at room temperature (± 25°C). The dried seeds were then packaged in transparent glass, hermetically sealed and stored in a cold chamber, at a temperature of approximately 7° C until the beginning of the experiment (6 months).

Table 1. Maturation stages of *Passiflora cincinnata* and *Passiflora morifolia* fruits according to the number of days of the anthesis until the fruit harvest.

Maturation Stages	Number of days of the anthesis until the fruit harvest
Passiflora cincinnata	
Stage 1 – Green	43
Stage 2 – Breaker	53
Stage 3 – Ripe	63
Stage 4 - Overripe	73
Passiflora morifolia	
Stage 1 – Green	27
Stage 2 – Breaker	32
Stage 3 – Ripe	37
Stage 4 - Overripe	42

Determination of the humidity degree was completed according to the oven drying method (105 ± 3°C for 24 hours), proposed by Brasil (2009). Two samples of 20 seeds were used for each stage of the two *Passiflora* species after the storage. The results were expressed in percentage.

Seeds of the two studied species were fixed in transparent sheets with double-sided tape in a completely randomized design, with four repetitions of 50 seeds for each maturation stage. To obtain the radiography images, the seeds exposed in the transparent sheets were submitted to the X-ray exposition, through the Faxitron MX-20 DC 12 equipment; with automatic adjustment of the exposition time and radiation intensity. After the capture of the images, the seeds were classified in full seeds (fs) and empty seeds (es).

The capture of the images was made in high resolution, through the GroundEye® equipment, which is an information capture machine composed of a conveyor belt and realtime analysis through video, allied to a software (TBIT, 2014). To capture the images, four repetitions of 50 seeds exposed in the transparent sheets with double-sided tape were placed on a reading tray (transparent acrylic tray of the equipment), making four repetitions for each maturation stage of the seeds of the two species.

Following the capture of the seed images by GroundEye®, the software generated spreadsheets with the captured information. In this study, ten descriptors of color and seven of geometry were evaluated. The ten descriptors related to color were: the color dominance: black, sky blue color, cyan blue color, dark grey, olive green, orange, yellow, and red, beyond the intensity and brightness of the colors. In geometry, the seven descriptors were: Area, Circularity, Shape Sphericity, Maximum diameter, Minimum diameter, Perimeter, and Fine-tuning. All data were calculated automatically by the software (TBIT, 2014).

The seeds from X-ray tests and image analysis were utilized for germination test mounting, being previously employed overcoming dormancy treatment. The employed overcoming dormancy used the following protocols:

a) *P. cincinnata*: The seeds were imbibed in Promalin® solution (GA4+7 + N-(phenylmethyl)–aminopurine) at the concentration of 0.90% for 12 hours in BOD (biochemical oxygen demand) incubator at 30º C, in light absence (LUZ et al. 2021a).

b) *P. morifolia*: The seeds were imbibed in GA3 (gibberellic acid) solution, at the concentration of

DOI: http://dx.doi.org/10.24021/raac.v20i1.7443 V. 20, N. 1 (2023)

1000 ppm (1000 mg L-1) for 5 hours in BOD (biochemical oxygen demand) incubator at 30º C, in light absence (MAROSTEGA *el al.*, 2017).

The seeds previously treated to overcome dormancy were used for the germination test, coming from X-ray tests and image analysis, maintaining the same treatments, with completely randomized design, four repetitions of 50 seeds for each treatment. The seeds were arranged in transparent acrylic boxes (Gerbox), using two sheets of blotting paper humidified with distilled water as a germination medium, in a proportion of two and a half times the mass of the dry paper. The boxes were packed in transparent polyethylene bags, to humidity maintenance, and maintained in germination chamber (BOD), with temperature alternation of 20 – 30°C in light absence for 30 days.

Evaluation of the germination test proceeded according to the Rules for seed analysis. During the 30 days of experiment, the amount of the seeds that emitted radicle was annotated, considering germinated seed those that presented tegmental rupture and emission of radicle with at least 2 mm in length (BRASIL, 2009).

All germination variables of the present study were calculated using the software GerminaQuant 1.0 (MARQUES *et al.*, 2015), where, at the end of the observations, were calculated germinability (%) (Transformed in arcsine %), the ATG – average time of germination, the ASG - average speed of germination (MAGUIRE, 1962), U – germination uncertainty, variable that indicates if the process happened or not (LABOURIAU; VALADARES, 1976), and Z – germination synchrony, which indicates when at least two seeds germinate together (PRIMACK, 1980).

The obtained data were submitted to analysis of variance and the averages were compared by Tukey test at 5% of probability through the computer software SISVAR (FERREIRA, 2011).

A correlation between the seed classes was completed, observed on the X-ray tests and image analysis with the germination data, presented by the germination test, considering the variables: full seed percentage; empty seed percentage, color dominance: black, sky blue color, cyan blue color, dark grey, olive green, orange, red, geometry data: Area, circularity, shape sphericity, maximum diameter, minimum diameter, perimeter and fine-tuning, color intensity, brightness, and germination. The software Microsoft Excel was adopted as a statistic tool to analyze Person linear correlation.

3. RESULTS AND DISCUSSION

The humidity degree, because of the fruit collection, were 9.55; 9.98; 9.29 e 9.62%, respectively to the stages 1, 2, 3, and 4 of *P. cincinnata* seeds. For *P. morifolia*, the values were 12.83; 13.77; 12.59 e 13.21% equally for stages 1, 2, 3, and 4. For both species, the humidity degree was determined after storage. The maturation stages were based on the number of days after flower anthesis, being for *P. cincinnata* 43, 53, 63 and 73 days and for *P. morifolia* 27, 32, 37 and 42 days. It was observed that there was a low variation of the humidity values in the function of maturation stages of fruits.

The X-ray exposition of the seeds of Passiflora species, in radiation intensity of 35 kV for 14.1 seconds, was the ideal condition for a great visualization of the seed intern morphology. In X-ray tests, image quality is directly related to radiation intensity and exposition time.

The exam of images of *P. cincinnata* and *P. morifolia* seeds, obtained by X-ray tests, allowed to evaluate seed intern conditions and, following the Rules for seed analysis (BRASIL, 2009), it was possible to classify the seeds in “full seed” or “empty seed”, according to figure 2.

DOI: http://dx.doi.org/10.24021/raac.v20i1.7443 V. 20, N. 1 (2023)

A significant difference between the categories “full” and “empty” was observed, classified by X-ray test and maturation stages of *P. cincinnata* and *P. morifolia* seeds. On table 2, percentages of viable and not-viable seeds are observed, being full seeds considered viable and empty seeds considered inviable.

According to table 2, from images radiographed by x-ray test is verified that stages 2 and 4 were higher than stage 1 for both species, referring to the percentage of full seeds. The allotments with a higher percentage of full seeds are classified as superior to the other and can promote better results to the germination test. The result of this study was similar to the result found by Severiano et al. (2018), which evaluate the intern morphology of passion fruit (*Passiflora edulis*). Seeds in different maturation stages through x-ray test; the authors observed that seeds of ripe fruits presented a better result related to the proportion of full seed.

Table 2. *Passiflora cincinnata* and *Passiflora morifolia* seeds, in the function of the maturation stage of the fruits, obtained in the full and empty categories by x-ray tests.

Maturation stage of the fruits	X-Ray Classification	
	Full seeds (%)	Empty seeds (%)
Passiflora cincinnata		
Stage 1 – Green	94 B	6 B
Stage 2 – Breaker	98 A	2 A
Stage 3 – Ripe	96 AB	4 AB
Stage 4 - Overripe	98 A	2 A
CV (%)	1,96	32,49
Passiflora morifolia		
Stage 1 – Green	89 B	11 C
Stage 2 – Breaker	98 A	2 AB
Stage 3 – Ripe	94 AB	6 AB
Stage 4 - Overripe	99 A	1 A
CV (%)	3.24	31.67

Averages followed by the same vertical letter do not differ from each other by the tukey test at 5% probability.

The category of full seeds for all maturation stages of both species was the one that presented the highest percentage, between 94 and 98% for *P. cincinnata* and 89 and 99% for *P. morifolia*. These values were close to the values found by Severiano *et al.* (2018), which, when evaluating seed quality of passion fruit (*P. edulis*) submitted to different aryl removal methods, observed that most of the seeds were classified as “well-formed”, by the X-ray test, with averages higher than 96.5% of full seeds.

Several authors as Abud *et al*. (2018) and Arruda *et al.* (2016) related the X-ray image analysis with the identification of mechanical damages and the physiological quality of the seeds. The comparison of the full seed and totally formed categories has been demonstrated correlation with the germination test to many agricultural species, as passion fruit (*P. cincinnata*) (LUZ *et al.*, 2021a), papaya (DIAS *et al.*, 2014), sesame (*Sesamum indicum* L.) (NOGUEIRA FILHO *et al.*, 2017), moringa (*Moringa oleifera* Lam.) (NORONHA *et al.*, 2018), among others.

Regarding the image capture, the best settings found for image definition are described below.

The background color calibration used was CIELab model with matrix between .0 e 100.0; saturation between -30.9 and 29.1; and brightness of -30.9 a -4.5. The recognition parameter type

DOI: http://dx.doi.org/10.24021/raac.v20i1.7443 V. 20, N. 1 (2023)

were seeds in general, a background of interior fill selected, and the minimum size of discard object of .01 cm². Seeds analyzed by the software GroundEye® in this study were revised by a manual correction of the excess part of the seeds.

Figure 2. *Passiflora cincinnata* and *Passiflora morifolia* seeds classified through X-ray image analysis. A1 and A2 – Full seed of *Passiflora cincinnata* and *Passiflora morifolia*, respectively. B1 and B2 – Empty seed of *Passiflora cincinnata* and *Passiflora morifolia*, respectively.

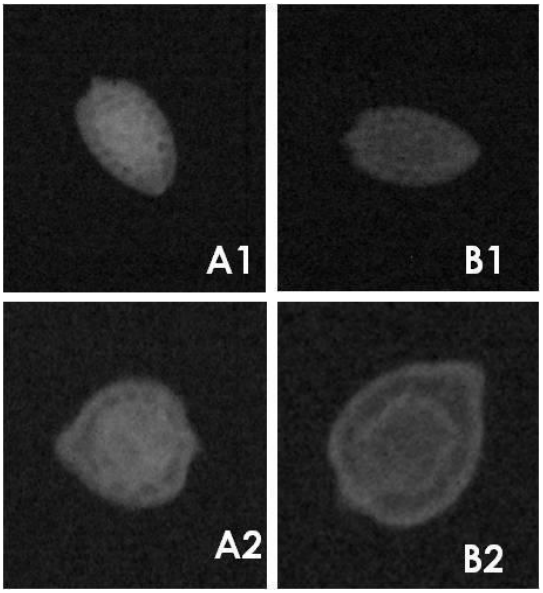


Table 3 presents the results of color classification of the seeds of *P. cincinnata* in the function of four maturation stages of the fruits, obtained by the software GroundEye®. It is possible to observe that the seeds obtained from fruits in advanced maturation stages (stages 3 and 4) presented a higher percentage of black color when compared to seeds on stages 1 and 2.

Averages followed by the same vertical letter do not differ from each other by the Tukey test at 5% probability.The percentage of the colors orange and yellow decreases and the percentage of dark grey color increased according to the progress of the maturation stage.

Both the intensity and brightness of the colors decreased according to the increase of the maturation time of the *P. cincinnata* fruits, where a high value of intensity and brightness were verified on the maturation stage 1 (green fruits). According to the results, the efficiency of the GroundEye® equipment to detect the dominance of present colors on the seeds of *P. cincinnata* fruits collected in different maturation stages is noticed.

Luz *et al.* (2021b) concluded that the GroundEye® system allows establishing parameters of Passiflora seed lots according to the fruit maturation stages, and can be highlighted as a quick vigor test and as reliable as traditional tests. Other authors as Rocha *et al.* (2015) concluded that image analysis is a viable, innovative, and promisor technique to evaluate the vigor of sunflower seeds (*Helianthus annus L.*).

In relation to the geometry characteristics of the studied seeds, the software measured the maximum diameter on the vertical and minimum diameter on the horizontal of each seed, generating a report of averages of each maturation stages of fruits. The analyses results indicated that maximum diameter between maturation stages do not differ statistically, with averages

DOI: http://dx.doi.org/10.24021/raac.v20i1.7443 V. 20, N. 1 (2023)

between 0.63 e 0.64 cm. For minimum diameter and area, stage 3 presents a higher average value, 0.36 cm e 0.18 cm2, respectively (Table 4).

In the function of maturation stages of *P. cincinnata* fruits, the variables: circularity, sphericity, perimeter and fine-tuning do not differ.

In general, *P. cincinnata* seeds from ripe fruits (stage 3) presented better results associated with the geometry, related to minimum diameter and area of the seeds.

On the quantification of descriptors of *P. morifolia* seeds color predominance, a higher contribution of the colors black, orange, and yellow was noticed, where the percentages vary between maturation stages of the fruits (Table 5).

On maturation stage 4, seeds presented 64.42% of the black color predominance, differing from the seeds of stages 1, 2 and 3, indicating that the more advanced is maturation, higher the black color occurrence.

Averages followed by the same vertical letter do not differ from each other by the Tukey test at 5% probability. Through the differences between maturation stages, it is possible to observe that the orange color represents 40.19% of the seed composition on stage 2. The yellow color presented the higher percentage on stage 1 (17.59%) and, the higher is the maturation stage, the lower is the percentage of this color on the seed constitution. Sky blue, cyan blue, dark grey, olive green, and, red colors do not represent significant percentage on the seed color constitution, and do not differ between the maturation stages of the fruits, except for the dark grey color that corresponded to 2.95% in seed in stage 1, and the larger is maturation stage, the lower is percentage of this color on seed constitution.

The intensity of the colors does not vary between maturation stages of the fruits. however, brightness was influenced by the stages, considering that stage 2 presented brighter seeds, followed by stage 1.

From the geometrical analysis, it is possible to observe, on *P. morifolia* seeds, that stage 1 (green) was superior in maximum diameter (0.51 cm). Stage 3 (ripe) presented the higher minimum diameter (0.36 cm²) (Table 6).

For circularity variable, is assumed value 1 for circular objects and lower than 1 for objects that distance from the circular format. Thus, maturation stages that present circularity values of the seeds close to 1, were more circular. Therefore, is verified on table 6 that seeds on stage 3 (seeds from ripe fruits) and stage 4 (seeds from overripe fruits) were the more circular ones, however, stage 4 does not differ from stage 2. Contrasting results were found to sphericity variable, because stage 1 presented better results, differing from stage 3. Regarding the area of the seeds (cm2), it was verified a smaller area for seeds on maturation stage 4 (overripe). To the variable perimeter, seeds on stage 1 presented higher values considering the other maturation stages of the fruits. Pádua *et al*. (2010) affirm that the size of the seeds, in many species, is a physiologic quality indicative, in other words, the higher is the seed, the higher is the physiologic quality. The larger seeds or those with greater density are those that normally have well-formed embryos and with greater amounts of reserves, potentially being the most vigorous (CARVALHO; NAKAGAWA, 2000).

In relation to the fine-tuning variable (Table 6), stage 3 presented better results, differing when compared to maturation stage 1. The geometry variables of the seeds are useful to help on the evaluation of existent variability between maturation stages of the fruits because besides they are accurate, they can be evaluated fast and effectively.

In general, seeds from ripe fruits (stage 3) presented better results related to the geometry. Size and form values of seeds are particular characteristics of each species, genetically defined,

DOI: http://dx.doi.org/10.24021/raac.v20i1.7443 V. 20, N. 1 (2023)

which can suffer environmental influence during and after formation period and storage, influencing other physic proprieties.

DOI: http://dx.doi.org/10.24021/raac.v20i1.7443 V. 20, N. 1 (2023)

Table 3. Characteristics of predominance of evaluated color (%), intensity, and color brightness. Classification of the seed colors of *Passiflora cincinnata*, collected from fruits in four maturation stages, according to the software GroundEye®.

	Characteristics of predominance of evaluated color (%), Intensity, and Color brightness								
Maturation stages of the fruits	Black	Sky blue color	Cyan blue color	Dark grey	Olive green	Orange	Yellow	Intensity	Bightness
Stage 1 – Green	34,21 C	0,00 A	0,00 A	0,39 B	0,03 A	24,62 A	40,73 A	0,23 A	0,28 A
Stage 2 – Breaker	65,18 B	0,01 A	0,01A	0,56 AB	0,02 A	13,98 B	20,21 B	0,19 B	0,23 B
Stage 3 – Ripe	74,30 A	0,00 A	0,01 A	0,60 AB	0,01 A	14,03 B	11,03 C	0,18 C	0,21 C
Stage 4 - Overripe	74,31 A	0,00 A	0,00 A	0,69 A	0,01 A	14,07 B	10,89 C	0,17 C	0,21 C
CV (%)	5,55	0,90	0,97	23,91	1,61	13,18	8,21	2,46	2,61

Averages followed by the same vertical letter do not differ from each other by the Tukey test at 5% probability.
Predominance variables of the colors: sky blue color, cyan blue color, oliver green were transformed in $(x + 0,5)^{0,5}$.

Tabela 4. Maximum diameter, Minimum diameter, Circularity, Shape Sphericity, Area, Perimeter, and Fine-tuning of *Passiflora cincinnata* seeds, in the function of four maturation stages of the fruits.

Maturation stages of the fruits	Max. diameter (cm)	Min. diameter (cm)	Circularity	Sphericity	Área (cm²)	Perimeter (cm)	Fine-tuning
Stage 1 – Green	0,63 A	0,35 B	0,54 A	18,15 A	0,17 B	1,75 A	0,70 A
Stage 2 – Breaker	0,63 A	0,35 B	0,54 A	18,34 A	0,17 B	1,76 A	0,70 A
Stage 3 – Ripe	0,64 A	0,36 A	0,55 A	18,26 A	0,18 A	1,81 A	0,70 A
Stage 4 – Overripe	0,63 A	0,35 B	0,55 A	17,92 A	0,17 B	1,74 A	0,70 A
CV (%)	1,85	1,15	1,51	3,82	2,48	2,92	2,73

Averages followed by the same vertical letter do not differ from each other by the Tukey test at 5% probability.

DOI: http://dx.doi.org/10.24021/raac.v20i1.7443 V. 20, N. 1 (2023)

Table 5. Characteristics of the evaluated color predominance (%), Intensity, and Color brightness. Classification of colors of *Passiflora morifolia* seeds, collected from fruits in four maturation stages, according to the software GroundEye®.

Characteristics of predominance of evaluated color (%), Intensity, and Color brightness									
Maturation stages of the fruits	Black	Sky blue color	Cyan blue color	Dark grey	Olive green	Yellow	Red	Intensity	Brightness
Stage 1 – Green	52,28 D	0,01 A	0,01 A	2,95 A	0,05 A	17,59 A	0,12 A	0,22 A	0,25 B
Stage 2 – Breaker	47,00 C	0,00 A	0,00 A	1,91 B	0,01 AB	10,83 B	0,03 A	0,22 A	0,26 A
Stage 3 – Ripe	61,05 B	0,00 A	0,00 A	0,80 C	0,00 B	4,43 C	0,05 A	0,19 A	0,23 C
Stage 4 - Overripe	64,42 A	0,00 A	0,00 A	0,77 C	0,01 AB	4,36 C	0,02 A	0,19 A	0,23 C
CV (%)	2,16	0,70	0,62	22,99	2,09	8,12	6,52	0,94	1,04

Averages followed by the same vertical letter do not differ from each other by the Tukey test at 5% probability. The predominance variables of the colors: sky blue color, cyan blue color, olive green were transformed in $(x + 0,5)^{0,5}$.

Table 6. Maximum diameter, Minimum diameter, Circularity, Shape Sphericity, Area, Perimeter, and Fine-tuning of *Passiflora morifolia* seeds, in the function of four maturation stages of the fruits.

Maturation stages of the fruits	Max. diameter (cm)	Min. diameter (cm)	Circularity	Sphericity	Área (cm²)	Perimeter (cm)	Fine-tuning
Stage 1 – Green	0,51 A	0,35 B	0,64 C	17,51 A	0,13 A	1,51 A	0,73 B
Stage 2 – Breaker	0,49 BC	0,35 B	0,66 BC	16,72 AB	0,13 A	1,45 B	0,76 AB
Stage 3 – Ripe	0,50 AB	0,36 A	0,68 A	16,24 B	0,13 A	1,46 B	0,77 A
Stage 4 – Overripe	0,48 C	0,34 C	0,67 AB	16,90 AB	0,12 B	1,43 B	0,75 AB
CV (%)	1,16	0,71	1,32	2,28	2,05	1,22	1,57

Averages followed by the same vertical letter do not differ from each other by the Tukey test at 5% probability.

DOI: http://dx.doi.org/10.24021/raac.v20i1.7443 V. 20, N. 1 (2023)

Maturation stage of the fruits of *P. cincinnata* influenced on germinability, average time (ATG), average speed (ASG) and uncertainty of germination (U) of the seeds (Table 7), evidencing that seeds can be collected before they are ripe or even green, without losses to the germination of the seeds. In another hand, overripe fruits presented lower results to germination, ATG, and ASG. In contrast to the obtained results, According to Santos *et al.* (2016), fruits of *Passiflora alata*, *P. cincinnata*, *P. edulis* and *P. setacea* can be harvested in the mature and senescent stages in order to provide seeds for plant multiplication without harming emergence. Luz *et al.* (2021a) verified an increase in the percentage of germination and in the speed of germination in *P. cincinnata* seeds taken from mature and senescent fruits, observed that the studied stages (breaker, ripe, and overripe) do not influence emergency rate and emergency speed index analyzed.

Still on Table 7, is verified uncertainty and synchrony of germination values. For uncertainty, is noted that the behavior between maturation stages differed only for stage 4 (overripe), which obtained lower values to the analyzed variable. Considering these results, it is possible to assume that germination was irregular in *P. cincinnata* seeds because high values for Uncertainty was observed. Synchrony values for different maturation stages do not differ.

Table 7. Germinability (Germ%), Average Time of Germination (ATG), Average Speed of Germination (ASG), Uncertainity of Germination (U) and Synchrony of Germination (Z) of seeds in the function of 4 maturation stages of *Passiflora cincinnata* fruits.

Maturation stages of the fruits	Germ (%)	ATG (Days)	ASG	U (bits)	Z
Stage 1 – Green	60 A	11,31 A	0,0886 A	2,9895 B	0,1270 A
Stage 2 – Breaker	56 A	11,65 A	0,0859 A	3,3097 B	0,0778 A
Stage 3 – Ripe	57 A	10,40 A	0,0962 A	3,3360 B	0,0800 A
Stage 4 - Overripe	8 B	19,63 B	0,0536 B	1,5333 A	0,0535 A
CV (%)	15,33	19,49	9,77	13,96	67,28

Averages followed by the same vertical letter do not differ from each other by the Tukey test at 5% probability.

The maturation of *P. morifolia* fruits was accompanied by the percentage reduction of germinability and, consequently, by dormancy intensity. The highest percentage of germination was for seeds obtained on stages 1, 2, and 3 (green, breaker, and ripe, respectively). The percentages of 80 and 94% of germination for seeds on stages 1 and 2 show that seeds did not present physiologic immaturity on this phase (Table 8). Santos *et al.* (2016) observed that the maturation stages breaker, ripe, and overripe of *P. alata*, *P. cincinnata*, *P. edulis* and *P. setacea* fruits do not influence emergency rate and emergency speed index of these species.

DOI: http://dx.doi.org/10.24021/raac.v20i1.7443 V. 20, N. 1 (2023)

Table 8. Germinability (Germ%), Average Time of Germination (ATG), Average Speed of Germination (ASG), Uncertainity of Germination (U) and Synchrony of Germination (Z) of seeds in the function of 4 maturation stages of *Passiflora morifolia* fruits.

Maturation stage of the fruits	Germ (%)	ATG (days)	ASG	U (bits)	Z
Stage 1 – Green	80 A	6,04 A	0,1661 A	1,7468 A	0,3463 A
Stage 2 – Breaker	94 A	6,12 A	0,1636 A	1,8880 A	0,3137 A
Stage 3 – Ripe	78 A	6,52 A	0,1555 A	2,0129 A	0,3058 A
Stage 4 - Overripe	29 B	6,66 A	0,1506 A	1,9292 A	0,2318 A
CV (%)	11,71	9,63	8,95	17,77	26,00

Averages followed by the same vertical letter do not differ from each other by the Tukey test at 5% probability.

Average time of germination (ATG), average speed of germination (ASG), uncertainty (U), and synchrony (Z) were not influenced by maturation stages of the seeds.

The correlation matrix presented in table 9 pointed existent correlations between the analyzed variables. Regarding the predominance of the colors dark grey, olive green, and yellow, as well as intensity and brightness of the colors, significant differences were verified, at 5% probability, between germinability.

Table 9. Pearson correlation matrix between germinability and full seed percentage (% full seed), empty seed percentage (% empty seed), dominance of the colors: black, sky blue color, cyan blue color, dark grey, olive green, Orange, and yellow, area, circularity, spherecity, maximum diameter, minimum diameter, perimeter, fine-tuning, intensity, and brightness of *Passiflora cincinnata* seeds, submitted to X-ray test, image analysis and germination test

	Germinability
% Full seed	-0,3346ns
% Empty seed	0,3346ns
Black	-0,4495ns
Sky blue color	-0,1401ns
Cyan blue color	0,0342ns
Dark grey	-0,5235*
Olive green	0,4707*
Orange	0,3669ns
Yellow	0,4718*
Area	0,2065ns
Circularity	-0,2137ns
Sphericity	0,2744ns
Maximum diameter	0,3842ns
Minimum diameter	0,3395ns
Perimeter	0,3178ns
Fine-tuning	-0,2071ns
Intensity	0,4773*
Brightness	0,4892*

DOI: http://dx.doi.org/10.24021/raac.v20i1.7443 V. 20, N. 1 (2023)

* Significant at 5% probability by t test. ns not significant.

Among the correlations with the variable germinability, only the dark grey color had established a negative moderated correlation, where the higher is the predominance of dark grey color in the constitution of the seed, the lower is germinability.

For predominance variables of green olive and yellow colors, intensity and brightness of the colors established positive moderated correlation. That is, the more olive green and yellow the seeds present and the greater is intensity and brightness of the colors, the greater is germinability.

Therefore, is assumed by the obtained results from germination test and image analysis that the earlier is collected *P. cincinnata* fruits, that is, the stages: green, breaker, and ripe, the better is the germinability of the seeds. The Pearson correlation results corroborate to the performed tests.

In Table 10 is observed the correlation between germinability and other analyzed variables of *P. morifolia*. The correlations between germinability and dominance of the colors: black, dark grey, and yellow, area, maximum and minimum diameter, perimeter, intensity, and brightness were significant.

Germinability correlated with the area variable positively and strongly. That is, the higher is the area of the seed, the higher is germination percentage. For the variables maximum diameter, minimum diameter, and perimeter, germinability correlated positive and moderately, indicating that the higher are the values of geometry characteristics of *P. morifolia* seeds, the higher is germinability.

The correlation of germinability with the variables intensity and brightness of the colors is characterized as positive and strong. In this way, the more intense and bright are the colors, the higher are the germination rates.

According to the obtained results in this study, the use of X-ray test in Passiflora seeds is extremely promisor on detection of quality of the seeds, between maturation stages of the fruits, supporting on empty seeds separation, considering the inviable to use immediately or to storage.

GroundEye® system allows establishing allotment parameters of Passiflora seed in the function of maturation stages of the fruits that can be determined by color or geometry, highlighting as a fast vigor test and as trustable as the traditional tests.

Table 10. Pearson correlation matrix between germinability and full seed percentage (% full seed), empty seed percentage (% empty seed), dominance of the colors: black, sky blue color, cyan blue color, dark grey, olive green, Orange, yellow, and red, area, circularity, spherecity, maximum diameter, minimum diameter, perimeter, fine-tuning, intensity, and brightness of *Passiflora morifolia* seeds, submitted to X-ray test, image analysis and germination test

	Germinability
% Full seed	-0,3324ns
% Empty seed	0,3324ns
Black	-0,7738**
Sky blue color	0,1231ns

DOI: http://dx.doi.org/10.24021/raac.v20i1.7443 V. 20, N. 1 (2023)

Cyan blue color	0,2621ns
Dark grey	0,5074*
Olive green	0,1802ns
Orange	0,4273ns
Yellow	0,5042*
Red	0,2409ns
Area	0,9321**
Circularity	-0,1310ns
Sphericity	0,0195ns
Maximum diameter	0,6038*
Minimum diameter	0,5741*
Perimeter	0,4731*
Fine tuning	-0,0371ns
Intensity	0,7101**
Brightness	0,7407**

** * Significant at 1 and 5% probability by t test, respectively. ^{ns} not significant.

4. CONCLUSION

P. cincinnata and *P. morifolia* seeds can be collected from fruits on the stages green, breaker, and ripe without losses to the physiologic quality.

The x-ray test was efficient to classify full and empty seeds of *P. cincinnata* and *P. morifolia*.

For *P. cincinnata* seeds, seed lots with the highest index of seeds with a predominance of black color indicate seeds of lower germinability, while seeds with a predominance of olive green and yellow colors indicate greater germinability.

The seeds of the *P. morifolia*, seed lots with the highest index of seeds with a predominance of black color indicate seeds of lower germinability, while seeds with a predominance of dark grey and yellow colors indicate greater germinability.

5. REFERENCES

ABUD HF, CICERO SM, GOMES JUNIOR FG. Radiographic images and relationship of the internal morphology and physiological potential of broccoli seeds. **Acta Scientiarum**, v. 40, p. 1-9, 2018.

ARRUDA N, CICERO SM, GOMES-JUNIOR FG. Radiographic analysis to assess the seed structure of *Crotalaria juncea* L. **Journal of Seed Science**, v. 38, p. 1-8, 2016.

BATTILANI JL, SANTIAGO EF, DIAS ES. Morfologia de frutos, sementes, plântulas e plantas jovens de *Guibourtia hymenifolia* (Moric.) J. Leonard (Fabaceae). **Revista Árvore**, v. 35, p. 1089-1098, 2012.

BRASIL. **Regras para análise de sementes**. Ministério da Agricultura, Pecuária e Abastecimento, Brasília, Brasil: Coordenação de Laboratório Vegetal, Departamento de

Defesa Vegetal, 399 p, 2009.

CARVALHO NM, NAKAGAWA J. **Sementes: ciência, tecnologia e produção**. 4. ed. Jaboticabal: FUNEP, 2000, 588 p.

DIAS MA, DIAS DCFS, GOMES JUNIOR FG, CICERO SM. Morphological changes and quality of papaya seeds as correlated to their location within the fruit and ripening stages. **Idesia**, v. 32, p. 27-34, 2014.

FARIA A, PAIVA SOBRINHO S, OLIVEIRA AS, TAVARES AR, LUZ PB. Cryoprotectants and X-ray analysis on Passiflora seeds cryopreserved. **Scientia Plena**, v. 17, p. 1-7, 2021.

FERREIRA DF. Sisvar: a computer statistical analysis system. **Revista Ciência e Agrotecnologia**, v. 35, p. 1039-1042, 2011.

KOBORI NN, CÍCERO SM, MEDINA PF. Teste de raios-X na avaliação da qualidade de sementes de mamona. **Revista Brasileira de Sementes**, v. 34, p. 125-133, 2012.

LABOURIAU LG, VALADARES MEB. On the germination of seeds of *Calotropis procera* (Ait.) Ait. f. **Anais da Academia Brasileira de Ciências**, v. 48, p. 263-284, 1976.

LUZ PB, LIMA BFS, PAIVA SOBRINHO S, OLIVEIRA AS. Utilização de análise de imagem para avaliação da qualidade de sementes de *Passiflora cincinnata* (maracujá-do-mato). **Multitemas** (UCDB), v. 26, p. 123-139, 2021a.

LUZ PB, LIMA BFS, PAIVA SOBRINHO S, OLIVEIRA AS, LOFFLER JJ. Superação de dormência em sementes de *Passiflora alata* e *Passiflora cincinnata*. **Revista da Universidade Vale do Rio Verde**, v. 20, p. 01-11, 2021b.

MAGUIRE JD. Speed of germination - aid in selection and evaluation for seedling emergence and vigor. **Crop Science**, v. 2, p. 176-177, 1962.

MAROSTEGA TN, LUZ PB, TAVARES AR, NEVES LG, PAIVA SOBRINHO S. Methods of breaking seed dormancy for ornamental passion fruit species. **Ornamental Horticulture**, v. 23, p. 72-78, 2017.

MARQUES FRF, MEIADO MV, CASTRO NMCR, CAMPOS MLO, MENDES KR, SANTOS OO, POMPELLI MF. GerminaQuant: A new tool for germination measurements. **Journal of Seed Science**, v. 37, p. 248-255, 2015.

NEVES SMAS, NUNES MCM, NEVES RJ. Caracterização das condições climáticas de Cáceres/ MT – Brasil, no período de 1971 a 2009: subsídios às atividades agropecuárias e turísticas municipais. **Boletim Goiano de Geografia**, v. 31, p. 55–68, 2011.

NOGUEIRA FILHO FP, OLIVEIRA AB, PEREIRA MS, LOPES MFQ, SILVA RT. Effectivity of X-ray test to evaluate the physiological quality of sesame seeds due to fruits position at the plant. **Revista Brasileira de Ciências Agrárias**, v. 12, p. 435-440, 2017.

DOI: http://dx.doi.org/10.24021/raac.v20i1.7443 V. 20, N. 1 (2023)

NORONHA BG, MEDEIROS AD, PEREIRA MD. Avaliação da qualidade fisiológica de sementes de *Moringa oleifera* Lam. **Ciência Florestal**, v. 8, p. 393-402, 2018.

PÁDUA GP, ZITO RK, ARANTES NE, FRANÇA NETO JB. Influência do tamanho da semente na qualidade fisiológica e na produtividade da cultura da soja. **Revista Brasileira de Sementes**, v. 32, p. 09-16, 2010.

PINTO CAG, CARVALHO MLM, ANDRADE DB, LEITE ER, CHALFOUN I. Image analysis in the evaluation of the physiological potential of maize seeds. **Revista Ciência Agronômica**, v. 46, p. 319-328, 2015.

PRIMACK RB. Variation in the phenology of natural populations of montane shrubs in New Zealand. **Journal of Ecology**, v. 68, p. 849-862, 1980.

ROCHA CRM, SILVA VN, CÍCERO SM. Avaliação do vigor de sementes de girassol por meio de análise de imagens de plântulas. **Revista Ciência Rural**, v. 45, p. 970-976, 2015.

SANTOS CHB, CRUZ NETO AJ, JUNGHANS TB, JESUS ON, GIRARDI EA. Estádio de maturação de frutos e influência de ácido giberélico na emergência e crescimento de *Passiflora* spp. **Revista Ciência Agronômica**, v. 47, p. 481-490, 2016.

SEVERIANO RL, PINHEIRO PR, GOMES JUNIOR FG, MEDEIROS AD, PEREIRA MD. X-ray test on passion fruit submitted to different aryl removal methods. **Comunicata Scientiae**, v. 9, p. 356-362, 2018.

TBIT. **Manual do Usuário SAS – Sistema de Análise de Sementes**. 1 ed. Lavras, MG, Brasil, 152 p, 2014.

DOI: http://dx.doi.org/10.24021/raac.v20i1.7443 V. 20, N. 1 (2023)