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# Physiological maturity of pitahaya (*Hylocereus undatus*) seeds and its correlation with fruit equatorial diameter

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# Abstract

Determining seeds physiological maturity is extremely important for all species because this knowledge facilitates harvest planning and quality control. The goal of the present study was to determine the physiological maturity point for pitahaya seeds (Hylocereus undatus) on the basis of seed maturation indices and to correlate this timing with the fruit equatorial diameter. Flowers were manually pollinated during the night and labeled at anthesis. The fruit collection began 21 days later and was performed for 12 days, with a daily harvest of four fruit. A completely randomized experimental design was used. First, the fruit equatorial diameter (FED) was measured, and then, to determine the point of physiological maturity, the seeds were extracted and the seed dry weight (DW), moisture content (MC), percentage germination (PG), germination speed index (GSI), mean germination time (MGT), and electrical conductivity (EC) were quantified, as were the root length (RL), shoot length (SL), and seedling dry weight (SDW). The seed maturation indices were subjected to an analysis of variance, and a polynomial regression analysis of the effect of the number of days after anthesis (DAA) and a Pearson correlation were also performed ( $p \le 0.05$ ). The physiological maturity of pitahaya seeds was reached between 28 and 31 DAA. Their highest DW and lowest MC were observed at 28 DAA. At 29 DAA, the reorganization of the membrane system was favored, as indicated by the EC. The highest RL was observed at 30 DAA, with the highest PG and SL occurring at 31 DAA. The FED was an efficient indicator of seed physiological maturity. The GSI was the maturation index that was more strongly associated with the remaining analyzed indices. Thus, when the aim is to obtain pitahaya seed of high physiological quality, the pitahaya fruit harvest should take place between 28 and 31 DAA. This period has been shown to be favorable in terms of seed maturation indices.

Keywords: Cactaceae, Maturation indices, Hylocereus undatus, Physiological quality, Vigor.

Abbreviations: DAA\_days after anthesis; DW\_seed dry weight; EC\_electrical conductivity; FED\_fruit equatorial diameter; GSI\_ germination speed index; MC\_moisture content; MGT\_mean germination time; PG\_percentage germination; RL\_root length; SL\_ shoot length; SDW\_seedling dry weight.

# Introduction

Pitahaya (*Hylocereus undatus* (Haworth) Britton & Rose) belongs to the Cactaceae family, and it originated from tropical and subtropical America. Its name means "scaly fruit" and refers to the pitahaya plant as well as its fruit (Zee et al., 2004; Tel-Zur et al., 2004). This species occupies a growing niche in the exotic fruit market, with increasing interest not only from consumers because of its organoleptic characteristics (Andrade et al., 2007; Silva et al. 2006) but also from fruit producers because of its added commercial value and its rusticity (Bastos et al., 2006; Ortiz-Hernández et al., 1999).

The pitahaya fruit is a berry, with succulent pulp and a white, red, or magenta color, depending on the species (Le Bellec et al., 2006; Livera-Muñoz et al., 2010; Lorenzi et al., 2006). The smooth and shiny seeds are distributed in large numbers throughout the fruit and have a diameter of approximately 3 mm, a dark color, and an obovate shape

(Crane and Balerdi, 2005; Le Bellec et al., 2006; Andrade et al., 2007). Pitahaya can be propagated through seeds or vegetative structures. Seeds are important for obtaining genetic information because of their variability; they exhibit desirable characteristics that may be used for genotype selection, such as those associated with yield, external appearance, pulp color and adaptation to different climate conditions (Andrade et al., 2008; Silva et al., 2011). In addition, the seeds exhibit high germination capacity, and, when compared with vegetative propagation, they experience a lower probability of disease transmission (Elobeidy, 2006; Verheij, 2005).

Determining when seeds reach physiological maturity is extremely important for all species because this information facilitates harvest planning and quality control, when considering that edaphoclimatic conditions may influence seed development (Alves et al., 2005; Iossi et al., 2007). The seed harvest season and the stage of maximum seed quality may be established through the study and determination of certain parameters, termed maturation indices, that are used to evaluate a range of morphological, physical, and physiological changes, such as moisture content, size, shape, color, fresh and dry weight, percentage germination, and vigor (Carvalho and Nakagawa, 2012; Marcos Filho, 2005; Piña-Rodrigues and Aguiar, 1993; Popinigis, 1985; Souza and Lima, 1985).

According to the literature, seed physiological maturity is reached when the seeds exhibit their maximum dry weights and a pronounced decrease in moisture content as well as visible changes in the external appearance of the fruits and seeds. Physiological maturity culminates in maximum seed germination capacity and vigor, when the seeds develop the capacity to perform their inherent physiological functions (Carvalho and Nakagawa, 2012; Popinigis, 1985).

Although the number of seed analysis studies on fruit species has increased, most of these studies lack basic information regarding ideal germination conditions (Oliveira et al., 2005). Determining when seeds reach physiological maturity is therefore essential to optimize their harvest and quality. The goal of the present study was to determine the physiological maturity point for pitahaya seeds (*H. undatus*) on the basis of seed maturation indices and to correlate this timing with the fruit equatorial diameter.

#### **Results and Discussion**

# Fruit equatorial diameter

The FED of pitahaya (*H. undatus*) varied linearly according to a regression analysis, with a significant increase from 7.0 to 7.9 cm at seed maturation (Fig. 2). In previous studies of pitahaya fruit maturation, Centurion Yah et al. (2008) observed a continuous increase during growth, with fruits reaching an equatorial diameter of 8.2 cm at 31 DAA, a value close to that obtained in the present study (7.9 cm), which is equal to that obtained by Osuna Enciso et al. (2007). Martínez-Chávez (2011) studied different *Hylocereus* spp. genotypes and reported pitahaya fruit equatorial diameters between 5.2 and 7.8 cm.

#### Seed dry weight and moisture content

A quadratic function was fitted to the behavior of DW and MC during seed maturation from 21 to 32 DAA, with the DW maximum and MC minimum values observed at 28 DAA (Fig. 3).

The DW was observed to increase as seed maturation advanced until 28 DAA (Fig. 3). In view of the fact that seeds are sinks for photosynthetic products, such as proteins, sugars, lipids, and other substances, plant-seed translocation will cease until a maximum is reached. According to the literature, the DW reaches its maximum value at physiological maturity, when the seed viability is highest and deterioration is lowest, and this measurement is therefore an efficient indicator of seed physiological maturity (Carvalho and Nakagawa, 2012).

The MC was inversely proportional with the DW, i.e., the initial seed water content was high and tended to decrease until 28 DAA. In accordance with the results obtained in the present study (Fig. 3), the seed MC has been observed to decrease with seed maturation in *Tibouchina granulosa* (Desr.) Cogn (Lopes et al., 2005), *Lycopersicon esculentum* Mill. (Vidigal et al., 2006), *Ricinus communis* L. (Silva et al., 2009), and *Jatropha curcas* L. (Santos et al., 2012).

The maintenance of a high MC in the seeds at the beginning of maturation is necessary for the translocation of photosynthetic products from the leaves of mother plants to the developing seeds, where they are used for seed formation and later as reserve compounds (Carvalho and Nakagawa, 2012). Because pitahaya seeds are protected by fleshy fruits throughout the entire maturation process, the decrease in seed MC is not pronounced. This moderate water loss occurs because the seeds contained in this type of fruit generally do not undergo a rapid dehydration phase, nor do they suffer great variation in the MC from changes in the relative humidity of the air.

Similar results were obtained during previous studies, with quadratic equations being fitted for the DW and MC during the seed maturation of *Bixa orellana* L. (Mendes et al., 2006), *Phoenix roebelenii* O'Brien (Iossi et al., 2007), and *Eugenia uniflora* L. (Avila et al., 2009). It can therefore be inferred that the observed behavior follows the expected pattern of seed development, i.e., after the initial growth phase (cell division), the water content is replaced with dry matter, causing the MC to decrease and the DW to increase. The decrease in the DW that was observed after the maximum point may be explained by desiccation or by the drying process that occurs during maturation, causing the values to decrease after the maximum size is reached (Castro et al., 2004).

# Percentage germination, germination speed index and mean germination time

The PG and the GSI fit a second-order polynomial regression model, whereas the MGT exhibited a linear behavior (Fig. 4). Germination was not observed for seeds from the first harvests. Germination then increased gradually, with intensification starting at 26 DAA, when seeds reached values close to 100% germination with the maximum point at 31 DAA, showing that the seed germination potential increases with maturation. The absence of seed germination in early harvests and the gradual increase of PG with maturation were also observed for Capsicum annuum L. (Sanchez et al., 1993), T. granulosa (Lopes et al., 2005), and L. esculentum (Vidigal et al., 2006). Negreiros et al. (2006) evaluated the influence of the maturation stage on the seed germination of Passiflora edulis f. flavicarpa and also observed increasing germination capacity over the experimental period.

The GSI was also best explained by a quadratic equation and increased with maturation (from 0.08 to 27.57), with its maximum point at 37 DAA (Fig. 4). Lopes et al. (2005) also observed an increasing GSI with increasing maturation time for *T. granulosa* seeds, with the highest levels, which were similar to those of the present study, observed for the latest harvests.

The MGT varied linearly with the harvest time, i.e., with advancing maturation, less time was needed for seeds to germinate. The germination time decreased from 10.73 to 3.77 days, indicating higher seed quality (Fig. 4). Valdes and Gray (1998) also observed significantly different mean germination times in *Lycopersicon lycopersicum* (L.) Karsten fruit of different ages and therefore at different stages of fruit maturation, with the MGT being higher in less mature seeds and decreasing with maturation.

#### Electrical conductivity

The EC decreased from 0.17 to 0.01  $\mu$ S cm<sup>-1</sup>, indicating that the reorganization of the membrane system was favored with



Fig 1. Daily minimum and maximum temperatures and the precipitation that occurred between the flowering and harvest of pitahaya fruit.



Fig 2. Fruit equatorial diameter of pitahaya between 21 and 32 days after anthesis.



Fig 3. Dry weight (A) and moisture content (B) of pitahaya seeds between 21 and 32 days after anthesis.

the advancement of seed physiological maturity because of lower solute leakage to the water solution, consequently resulting in higher seed vigor. A polynomial regression model was fitted to the EC data, and the model indicated that the EC reached its minimum value at 29 DAA (Fig. 5). The EC has been previously observed to decrease with seed maturation in the seeds of *L. esculentum* (Vidigal et al., 2006) and *Cucurbita moschata* Duch. (Neto et al., 2012), indicating that their cell membranes are satisfactorily organized by the time of fruit harvest.

Demir and Ellis (1992) observed a higher EC, i.e., lower vigor, for *L. esculentum* seeds harvested at 75 DAA than for seeds harvested at 45, 55 and 65 DAA, and physiological maturity may have been reached at earlier times.

# Root length, shoot length and seedling dry weight

The RL and SL ranged from 0.01 to 0.61 cm and from 0.1 to 1.9 cm, respectively, and they fit quadratic functions with similar trends, i.e., increasing over the experimental period

until reaching comparable maximum values at 30 and 31 DAA, respectively (Fig. 6). Firmino et al. (1996) reported similar results for the *Torresia acreana* Ducke, for which the RL and SL of seedlings that originated from the seeds that were harvested from unripe fruits exhibited the shortest lengths, indicating that these seeds exhibited slightly lower vigor than seeds that were closer to physiological maturity.

Braga Jr. (2009) studied the maturation of *Ricinus communis* L. and found that the seedling RL data fit a quadratic model, increasing until 21 DAA and linearly decreasing after that time point. It should be highlighted that the maximum point found by this author was observed considerably earlier than the time of seed physiological maturity, which occurred at 42 DAA.

Alexandre et al. (2006) studied three maturation stages (stage 1 was characterized by firm fruit and partial maturity, stage 2 consisted of firm fruit and maturity, and stage 3 involved fruits lacking in firmness that were collected from the ground) of *Myrciaria jaboticaba* Berg. seeds and observed an increased RL for firm and mature fruit. However, the RL



Fig 4. Percentage germination (A), germination speed index (B), and mean germination time (C) of pitahaya seeds between 21 and 32 days after anthesis.



Fig 5. Electrical conductivity of pitahaya seeds between 21 and 32 days after anthesis.



Fig 6. Root length and shoot length (A) and seedling dry weight (B) of pitahaya between 21 and 32 days after anthesis.

decreased for fruit that were lacking in firmness and were collected from the ground, again indicating the tendency of the seedlings to exhibit a longer RL until seed physiological maturity is reached, with a subsequent loss of vigor. The authors did not observe significant differences in the SL, unlike the present study, in which the seedling SL was observed to increase with seed maturation (Fig. 6). The resulting regression curve showed that the SDW also increased, which is in accordance with the RL and SL results (Fig. 6).

**Table 1.** Pearson correlation coefficients ( $\rho$ ) and their probabilities (p) for pairs of the following pitahaya variables as measured from 21 to 32 days after anthesis (DAA): fruit equatorial diameter (FED), seed dry weight (DW), moisture content (MC), percentage germination (PG), germination speed index (GSI), mean germination time (MGT), electrical conductivity (EC), root length (RL), shoot length (SL), and seedling dry weight (SDW).

Variables	FED	DW	MC	PG	GSI	MGT	EC	RL	SL	SDW
FED	ρ=1.00	$\rho = 0.80^{*}$	ρ=-0.29	ρ=0.88 <sup>*</sup>	ρ=0.85*	ρ=-0.61 <sup>*</sup>	ρ=-0.85*	ρ=0.83*	ρ=0.88*	ρ=0.85*
	<i>p</i> =	p=0.001	p=0.34	p=0.0002	p=0.0004	<i>p</i> =0.03	p=0.0004	<i>p</i> =0.0007	p=0.0001	p=0.0003
DW		ρ=1.00	ρ=-0.68*	ρ=0.84*	ρ=0.81*	ρ=-0.45	ρ=-0.94*	ρ=0.88*	$\rho = 0.87^{*}$	ρ=0.87*
		<i>p</i> =	p=0.01	p=0.0005	p=0.001	<i>p</i> =0.13	p=<0.0001	p=0.0001	p=0.0002	p=0.0002
MC			ρ=0.00	ρ=-0.42	ρ=-0.37	ρ=-0.02	ρ=0.55*	ρ=-0.50	ρ=-0.45	ρ=-0.49
			<i>p</i> =	<i>p</i> =0.16	p=0.22	p=0.94	p=0.05	p=0.09	p=0.13	p=0.10
PG				ρ=1.00	ρ=0.97*	ρ=-0.75*	ρ=-0.89*	ρ=0.98*	ρ=0.99*	ρ=0.98*
				<i>p</i> =	p = < 0.0001	p=0.004	p = < 0.0001	p = < 0.0001	p = < 0.0001	p = < 0.0001
GSI					ρ=1.00	ρ=-0.83*	ρ=-0.85*	ρ=0.94*	ρ=0.97*	ρ=0.96*
					<i>p</i> =	<i>p</i> =0.0007	p=0.0004	p=<0.0001	p = < 0.0001	p = < 0.0001
MGT						ρ=1.00	ρ=0.49	ρ=-0.66*	ρ=-0.73*	ρ=-0.74*
						<i>p</i> =	p=0.10	p=0.01	p=0.006	p=0.005
EC							ρ=1.00	ρ=-0.93*	ρ=-0.90*	ρ=-0.87 <sup>**</sup>
							<i>p</i> =	p = < 0.0001	p = < 0.0001	p=0.0002
RL								ρ=1.00	ρ=0.98*	ρ=0.96*
								<i>p</i> =	p = < 0.0001	p = < 0.0001
SL									ρ=1.00	ρ=0.99*
									<i>p</i> =	p = < 0.0001
SDW										ρ=1.00
										<i>p</i> =

\* $p \le 0.05$  by t-test for the Pearson correlation coefficient.

Sader and Silveira (1988) evaluated the physiological maturity of *Helianthus annuus* L. seeds and observed that the SDW tended to increase with maturation. However, the highest values were observed before physiological maturity, although no significant differences were observed until that stage. Corvello et al. (1999) studied the evolution of seed maturation in *Cedrela fissilis* Vell. and observed that the SDW increased until 35 weeks after anthesis (WAA), even after the seeds reached physiological maturity, which occurred between 29 and 31 WAA.

#### Correlation analysis

The statistically significant correlations between the variables that showed linear dependence were considered to be strong when the correlation coefficient  $(\rho)$  was equal to or exceeded 0.80, and those with lower values were classified as weak correlations (Ortiz et al., 2014) (Table 1). The FED showed strong, linear, positive correlations with the DW, G, GSI, RL, SL, and SDW and a negative correlation with the EC. The FED was therefore correlated with most of the variables that were analyzed as maturation indices for pitahaya seeds, indicating that this measurement may be used as an indicator of seed physiological maturity for this species, especially considering that it is a variable that can be easily and nondestructively evaluated by producers. In accordance with the present study, Avila et al. (2009) observed that the physiological maturity of E. uniflora seeds was reached when the FED reached its maximum, showing the efficiency of this variable for the identification of seed physiological maturity. The DW, PG, GSI, EC, RL, SL and SDW were also considered efficient indicators of the physiological maturity of H. undatus seeds because they exhibited a strong linear dependence on six other variables, or seven other variables in the case of the GSI (Table 1).

The DW was strongly, linearly, and positively correlated with the PG, GSI, RL, SL, and SDW, indicating that these variables increased proportionally, and the DW negatively correlated with the EC, i.e., the DW increased in proportion with the decreases in the EC (Table 1). The DW was also reported to be a significant indicator of seed physiological maturity in Glycine max L. Merr. (Crookston and Hill, 1978). However, Guimarães et al. (1998) reported that this parameter can not be used effectively as a maturation index for Zinnia elegans Jacq.. Mendes et al. (2006) reported that the DW was significantly and positively correlated with the PG (p=0.90) in Bixa orellana L. seeds, and these variables were also shown to be strongly and linearly correlated in the present study (p=0.84, Table 1). The same authors also reported that the maximum DW coincided with peaks in germination and vigor and that the DW therefore efficiently indicated the physiological maturity point. However, for certain species, maximum seed vigor and germination do not coincide with the highest dry weight, indicating that different quality characteristics continue to develop after the seeds reach their peak dry weights (Ellis et al., 1992; Lima, 1999). The PG was also negatively correlated with the EC and positively correlated with the GSI, RL, SL, SDW, and DW (Table 1). Iossi et al. (2007) observed similar results for P. roebelenii, confirming the coincidence of the maximum values of the seed dry weight, vigor, and percentage germination and establishing a correlation between these characteristics to determine the point of seed physiological maturity. The GSI exhibited a strong, linear, negative correlation with the EC and MGT and was positively correlated with the RL, SL, SDW, DW and PG (Table 1). Lopes et al. (2005) observed that the GSI was strongly and positively correlated with the PG in T. granulosa seeds ( $\rho$ =0.98), which is in accordance with the present study  $(\rho=0.97)$ . The EC was negatively correlated with the RL, SL, SDW, DW, PG, and GSI, whereas the RL and SL were strongly, linearly, and positively correlated with each other and with the SDW, DW, PG and GSI. No other significant, strong correlations were observed between the measured variables, including the MC and MGT. The MC and MGT were the only variables exhibiting weak linear correlations, with the first being positively correlated with the EC and the second negatively correlated with the RL, SL, and DW (Table 1).

However, Silva et al. (2009) observed that the seed MC was an efficient parameter for the identification in *R. communis* seeds at harvest point, especially when used together with the fruit and seed colors.

However, correlation testing should not be used alone to identify the point of seed physiological maturity because this approach may result in incomplete or incorrect information; significant correlations indicate similar trends in variations between two parameters but do not necessarily indicate the occurrence of seed physiological maturity in an accurate manner.

# **Materials and Methods**

# Study area characterization and plant material

This study was performed at the State University of Londrina (Universidade Estadual de Londrina-UEL) in the State of Paraná, Brazil, between January and May 2012. Seeds were obtained from the fruit of white-fleshed pitahaya (*Hylocereus undatus*) mother plants that were approximately 10 years old and had been grown in the experimental area of the UEL Agronomy Department at 23°23'S, 51°11'W, at a mean elevation of 566 m. The pitahaya plants were cultivated in an area with soil classified as a latosolic Eutroferric Red Nitrosol (USDA Alfisol; EMBRAPA, 2013), with 2.0 x 3.0 m spacing between the plants, which were grown vertically on 2.5-m-high trellises with two plants on each trellis.

# Treatments

For the seed physiological maturity study, flowers were manually pollinated during the night and labeled at anthesis. Fruit harvest began 21 days later and was performed for 12 days, with four fruits harvested per day. A completely randomized experimental design was used. Four subsamples were obtained from each harvested fruit, for a total of 16 replicates per treatment.

#### Environmental conditions

The minimum and maximum temperatures and precipitation in the experimental area were recorded during the entire experimental period by using a datalogger (HT-500<sup>®</sup>, Instrutherm Instrumentos de Medição Ltda, São Paulo, Brazil) (Fig. 1).

# Variables measured

The fruit was harvested in the morning and immediately taken to the UEL Laboratory of Seed Technology and Production to measure the given parameters. The FED was measured in cm by using an analog caliper to determine its correlation with the seed maturation indices that were analyzed. The fruit pulp was manually extracted with a spoon, placed in a 2-L beaker with 1 L of an aqueous sucrose solution (25 g  $L^{-1}$ ), and left to stand for 48 h at ambient temperature to promote fermentation for facilitating seed extraction. The pulp-sucrose suspension was then sieved under running water to eliminate the pulp remains and recover the seeds. The seeds were subsequently placed on paper and dried in the shade at ambient temperature for 48 h (Ortiz et al., 2015). The DW and MC were measured in replicates composed of 100 seeds. The seeds were dried in an oven dryer at 105±3°C for 24 h and placed in a desiccator for approximately 2 h until cool. The dry weights were then measured with a digital analytical scale (AW 320<sup>®</sup>,

Shimadzu Corp., Kyoto, Japan) and are expressed in g (Brasil, 2009). The MC was calculated as the difference between the fresh and dry weights of the seeds in %. The PG was determined in replicates composed of 50 seeds. The seeds were placed in crystal polystyrene boxes (Gerbox<sup>®</sup>) lined with blotting paper that had been moistened with an amount of distilled water that was equivalent to two and onehalf times the substrate dry weight (Brasil, 2009). The germination test was conducted in a geminatation chamber with constant light at 25°C. The evaluations were performed daily for 19 days, by which time the germination had stabilized. The seeds were considered to have germinated when they exhibited a root length that was equal to or greater than 2 mm, and the results are expressed in %. Along with the germination test, the GSI was determined according to Maguire (1962), and the MGT was determined in days according to Lima et al. (2006), based on daily counts of germinated seeds. The EC was determined using replicates composed of 100 seeds. The seeds were placed in plastic cups containing 10 mL of distilled water and incubated in a germinator at 25°C. The measurements were performed after 24 h of imbibition, by using an EC meter (CD-21<sup>®</sup>, Digimed, São Paulo, Brazil). The data are expressed as mean values in  $\mu$ S cm<sup>-1</sup>. For the seedling length test, replicates of 25 seeds were used. The seeds were placed in plastic boxes (Gerbox<sup>®</sup>) lined with blotting paper that had been moistened with an amount of distilled water equivalent to two and one-half times the substrate dry weight (Brasil, 2009). The test was conducted in a germination chamber with constant light at 25°C. The RL and SL of all the normal seedlings were measured after 21 days, by using a graduated ruler. The lengths were averaged, and the mean values are expressed in cm. The seedlings were then placed in paper bags in an oven dryer at 65°C for 24 h, and the SDW was then measured with a digital analytical scale (AW 320<sup>®</sup>, Shimadzu Corp., Kyoto, Japan). The results are expressed in g seedling<sup>-1</sup>.

# Statistical analysis

The seed maturation indices were subjected to an analysis of variance. In addition, a polynomial regression analysis was performed to determine the effect of the number of days after anthesis (DAA), and a Pearson correlation analysis was also performed ( $p \le 0.05$ ).

# Conclusions

The physiological maturity of pitahaya seeds (Hylocereus undatus) was reached by between 28 and 31 days after anthesis (DAA). Their highest dry weight and lowest moisture content were observed at 28 DAA. The reorganization of the membrane system was favored at 29 DAA, as indicated by the electrical conductivity. The seedlings exhibited a higher root length at 30 DAA, and the highest percentage germination and shoot length were observed at 31 DAA. The fruit equatorial diameter was an efficient indicator of seed physiological maturity. The germination speed index was the maturation index that was more strongly associated with the remaining analyzed indices. Thus, when the aim is to obtain pitahaya seed of high physiological quality, the pitahaya fruit harvest should take place between 28 and 31 DAA; this period has been shown to be favorable in terms of seed maturation indices.

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