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Physical and physiological changes of *Crambe abyssinica* Hochst seed during maturation

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ABSTRACT. Determination of seed-maturation indicators enables the identification of the ideal moment for harvest to achieve the best production and conservation potential. Our objective here was to evaluate some physical and physiological changes of crambe (*Crambe abyssinica* Hochst) seeds as possible indicators of seed maturation. Crambe flowering was monitored in Dourados, Mato Grosso do Sul, Brazil. Plants were tagged, and 13, 22, 26, and 28 days after the initiation of flowering, the seeds were collected and following physical attributes evaluated: length, diameter, total mass, dry matter and water content. Physiological quality of the seeds was assessed using the germination test, by registering the percentage of normal seedlings and dormant seeds, immediately after each harvest, and again after six months of storage. The water-absorption curves were characterized as a function the seed-development stages. All physical attributes were observed to increase because of the accumulation of reserve substances during seed development, except for water content, which gradually decreased from 72.2% at the start of development to 29.5% at maturity. At 28 days after anthesis the germination percentage of crambe seeds at physiological maturity was only 17%, indicating that they became dormant while maturing. However, seed germination rate was 89% after six months of storage, indicating that dormancy was almost fully overcome after this period.

Keywords: Brassicaceae; germination; oilseeds; physiological maturity.

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Introduction

Mitigation of the effects of climate change by reducing emissions of greenhouse-gases is one of the driving forces of biofuel production in developing countries, where the promotion of bioenergy is a clear opportunity to associate economic growth with environmental pollution cleanup (Lambin & Meyfroidt, 2011; Popp, Lakner, Rákos, & Fári, 2014). There is admittedly, a growing need for the development of renewable energy sources with lower negative environmental impact than that of the traditional ones. This need has recently stimulated interest in the search for alternatives to petroleum-based fuels (Lambin & Meyfroidt, 2011).

Brazil has exceptional competitive advantages for the implementation of a bioenergy-based energy matrix. Among these advantages, the availability of large areas with adequate soil and climate conditions for the sustainable production of biomass deserves careful consideration (Bringezu et al., 2007). As for the different biomass production systems, among the various oilseed crops with interesting biofuel production characteristics, crambe (*Crambe abyssinica*), of the Brassicaceae family, is currently being extensively cultivated in tropical areas.

Crambe oil contains high levels of erucic acid, which is widely used to create chemical products, plastic bags, cosmetics, detergents and other chemical compounds for industry (Qi et al., 2018) and other electrical components that this crop and its derivatives continue to gain economic importance. Studies on crambe have intensified, aiming to develop technologies to increase the knowledge about this crop, from acquisition of high quality seeds to handling and production techniques, since it is known that the crop success starts with the use of high quality seeds and that previous knowledge of the characteristics required for seed maturation is fundamental.

The control of the seed morphogenesis stage, including early embryo development and patterning, the maturation stage, including embryo growth, accumulation of storage reserves, desiccation tolerance acquisition and entry into dormancy (Rodrigues & Miguel, 2017) are generally associated with seeds physical aspects changes. Initially, the size and weight of fruit and seed increase and the possible reason is that the main metabolism process occurring in fruit and seed in this phase is the conversion of nutrition and cell histological differentiation and development (Yu & Chen, 2012). Mature seeds are an ultimate physiological status that enables plants to endure extreme conditions such as high and low temperature, freezing and desiccation (Sano et al., 2016).

Since the identification of the moment when the seeds reach their maximum physiological potential must be a primary factor in the crop seed-production chain and a major determinant of seed vigor (Leprince, Pellizzaro, Berriri, & Buitink, 2016) and the techniques of crambe cultivation are not yet fully developed, studies are being performed to regularize the sourcing of high-quality mature seeds to establish an adequate high-yielding crop.

In view of the economic importance that crambe cultivation is rapidly achieving because its potential alternative use as a secondary crop (Soratto, Schlick, Fernandes, & Souza, 2013), we have focused to determine the moment at which the seeds reach the maturity required for establishing uniform plant stands and to evaluate the physical and physiological changes that integrate crambe seed maturation.

Material and methods

The plant material used in the study was the crambe (*Crambe abyssinica*) cultivar FMS Brilhante. The experiment was performed in a dystroferic red latosol with very high clay content at the experimental farm of the *Universidade Federal da Grande Dourados* (UFGD), in Dourados – MS, Brazil (22° 11' 44" S, 54° 56' 08" W) at 430 m asl. The climate in the region, according to the Köppen classification, is humid mesothermal, type Aw (Peel, Finlayson, & McMahon, 2007), with average annual temperature and precipitation varying from 20 to 24°C and from 1,250 to 1,500 mm, respectively.

The chemical analysis of the 0-0.20 m layer showed the following results: OM = 24.9 g dm⁻³; pH (CaCl₂) = 5.2; P (resin) = 10.3 mg dm⁻³; H+Al = 57.3 mmol_c dm⁻³; K⁺, Ca²⁺ and Mg²⁺ = 4.5; 15.5 and 57.3 mmol_c dm⁻³, respectively; V% = 59 and CTC = 139.5 mmol_cdm⁻³.

Sowing was mechanically performed on April 25, 2016 with 20 seeds m⁻¹ spaced 0.4 m⁻¹ between rows and the emergence occurred on May 02, 2016. Pests and diseases were controlled according to the technical recommendations for the crop.

Flowering was characterized when approximately 50% of the plants showed at least one flower at anthesis, 35 days after sowing. Flowers were tagged with colored plastic ribbon to differentiate them at anthesis. For the flowers tagged, harvest was performed in order to evaluate the seed development at 13, 22, 26, and 28 days after anthesis (DAA). Climactic data recorded during seed development is shown in Figure 1.

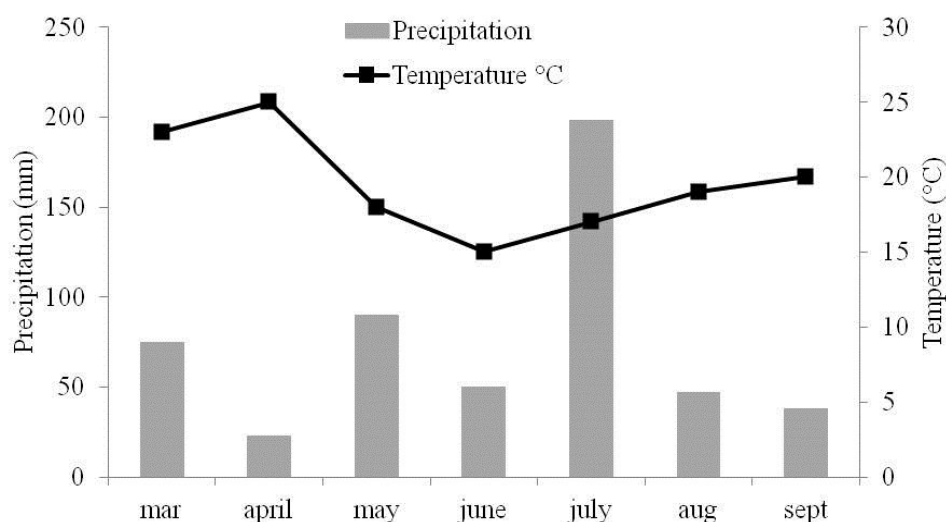


Figure 1. Monthly precipitation and average temperature during crambe seed harvest in 2016.

Source: Agrometeorological Station of the Agricultural Sciences Experimental Farm - UFGD, Dourados, MS, Brazil.

On each evaluation date, plants were manually harvested for manual threshing, and the seeds were submitted to the following evaluations:

Seed size and mass. Seed size was evaluated by determining seed length and diameter with a digital caliper (0.01 mm); individual seed mass was determined using a balance (0.0001 g). Seed length was measured as the distance between basal and apical regions, while seed diameter was determined at the middle point of the seed. Four samples of 25 seeds each were tested on each evaluation date.

Determination of water content. Seeds were oven dried at $105 \pm 3^\circ\text{C}$ for 24 hours, with four sub-samples per treatment (Brasil, 2009). Data were expressed as percentage on a wet weight basis. At the same time, the accumulation of dry matter in the seeds (g seed^{-1}) was determined.

Germination tests. Germination was tested using four samples of 25 seeds each. The seeds were placed on germitest paper inside plastic 'gerbox' containers, moistened with distilled water in the proportion of 2.5 times the mass of the dry paper, at 25°C in B.O.D. incubator, for a period of seven days. The percentages of normal seedlings and dormant seeds were evaluated (Brasil, 2009).

Seed storage. To evaluate the possibility of overcoming seed dormancy, the seeds remained wrapped inside a paper bag in a room at 25°C and 65% relative humidity (RU), for 180 days. After this period, seeds were subjected to the germination test and the percentage of normal seedlings and dormant seeds was calculated (Brasil, 2009).

Water absorption curve. Seeds were placed on petri dishes lined with two sheets of germitest paper soaked in an amount of distilled water equivalent to 2.5 times the mass of the dry paper and were kept in B.O.D. at 25°C (Brasil, 2009). Seeds were weighed individually using a precision scale (0.0001 g) every two hours following the start of the test, and until the emergence of the primary root. The test was performed using four replications with five seeds each, on each evaluation date.

Statistical analysis

The experiments were conducted following a completely randomized design with four replications. The data on physical and physiological attributes of seed quality at each stage of development were tested for normality and homogeneity of variance. Data were submitted to Anova and regression analyses at 5% level of probability using the statistical software - Sisvar (Ferreira, 2011).

Results and discussion

There was a significant effect of seed-developmental stage on seed diameter (Figure 2A) and length (Figure 2B). A linear increase in diameter was observed during seed development, whereby, 28 DAA seeds measured 2.91 cm (maximum length recorded 23.6 DAA was 2.90 cm), indicating development of internal attributes and accumulation of reserve substances in the seeds continued throughout the duration of seed development up to maturity.

Seed-development stage also had a significant effect on total seed mass, with a maximum mean of 0.36 g seed^{-1} on day 19.6 (Figure 2C). From this moment onwards, there was a reduction in seed mass that may be associated with the loss of moisture, with the minimum water content (31.7%) registered 26.5 DAA (Figure 2D), suggesting natural seed dehydration as maturation progressed; a typical behavior of orthodox seeds.

Seeds showed a total mean mass of 0.310 and 0.248 g, 14 and 28 DAA, respectively (Figure 2C); on the same dates, seed water content was 72.2% and 29.5%, respectively (Figure 2D), indicating that seeds still showed a high water content at harvesting.

Conversely, mean seed dry matter continued to increase throughout seed development (Figure 2E), peaking at $0.068 \text{ g seed}^{-1}$, which indicated an accumulation of reserve substances in the internal matrices of crambe seeds and that the total decrease in total seed mass (Figure 2C) can be fully explained by the reduction in water content that took place during the final stages of the seed maturation process. The variations that occurred over the experimental period indicate that the size of the seed can be considered as a physical trait that varies over the course of development of the crambe seeds. According to Marcos Filho (2015), the first stages of embryogenesis involve intensive cellular division and expansion; these events lead to a progressive increase in the size of the developing seed, peaking approximately halfway through the period of dry matter accumulation.

Seed development occurs through distinct phases, beginning with cellular division and accelerated mass gain, consisting mostly of water, and over time, the water content decreases with the synthesis and

accumulation of reserve substances (Bewley & Black, 1994; Marcos Filho, 2015). The reserve substances allocated in the crambe seed endosperm are lipids, proteins and soluble sugars (Oliveira et al., 2016). Concomitant with the reduction in seed water content, a reduction in total mass was also observed, just as the largest seed-water content was previously accompanied by greater seed total mass (Figures 2C and 2D).

During the natural drying phase that takes place in the embryo in the final stage of maturation, mechanisms promoting seed longevity in the dehydrated seed become activated; consequently, there is a reduction in seed-water content that needs to be precisely controlled to allow abscission from the fruits of the mother plant (Leprince et al., 2016). Although dry mass is a great indicator of physiological maturity, it must not be used in isolation as an indicative parameter, as some works have demonstrated that certain seeds undergo physiological and biochemical alterations, even after having reached maximum dry matter content (Carvalho & Nakagawa, 2012). Knowledge of the physical parameters involved in the process of crambe seed maturation is relevant, since, according to Oliveira et al. (2014), this species has an indeterminate growth habit, which means there are seeds at different stages of maturation within the same plant that influences the time of harvest, and, consequently, the physiological quality of the seeds.

Seed-development stage had a significant effect on the percentage of normal seedlings obtained, which increased linearly up to 28 DAA (Figure 3A). Although low germination results were observed for all evaluation dates. We verified that seed germination capacity increased during development; furthermore, the maximum amount of dry matter accumulation was concomitant with the maximum seed-germination rates recorded.

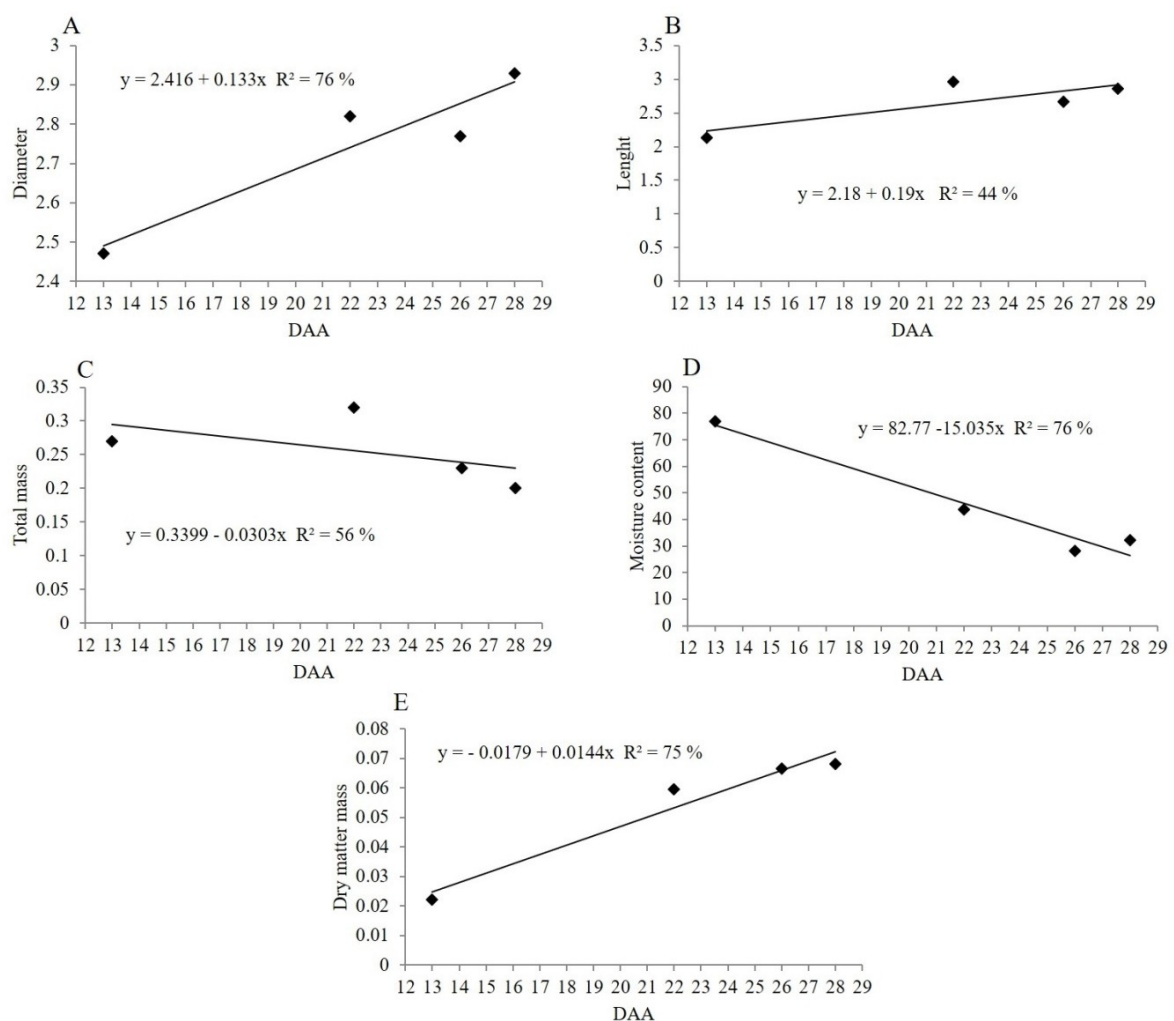


Figure 2. A - Diameter (mm); B - length (mm); C - total mass (g seed⁻¹); D - moisture content (%); and E - dry matter mass (g seed⁻¹) of crambe seeds at different developmental stages. DAA - days after anthesis.

Seeds obtained on the first harvesting date produced only 6% normal seedlings, i.e., less than half the percentage of normal seedlings obtained on the last harvest, 28 DAA (15%) (Figure 3A). This may be

attributed to physiological immaturity of the seed, as suggested by the fact that over the near three-week interval between the first and last evaluations, seed germination capacity increased significantly (Figure 3A). Even if seeds were dispersed at the same time, variations in seed size, shape, and other physical or biochemical characteristics can result in non-uniform germination. Although many of these characteristics are genetically determined, there is a strong adaptive relationship between these traits and local environmental conditions prevailing during seed maturation (Brancalion & Marcos Filho, 2008). In these experiments, we observed that the capacity for germination was gradually acquired throughout seed development, whereby, the first seed harvest produced only 6% normal seedlings; 28 DAA the seeds presented 15% normal seedlings (Figure 3A).

The low germination percentages observed in the seeds harvested between 14 and 26 DAA can be associated with the undetermined flowering habit that characterizes crambe crops, which can result in non-uniform development and post-harvest dormancy of the seeds (Oliveira et al., 2014). In this regard, although the capacity for germination is established during seed development, the low germination results indicated a deep state of dormancy of the harvested seeds (86%).

Conversely, seed-development stage had no significant effect on the percentage of dormant seeds, which reached 86%, 28 days after flowering, i.e., at maturity. After the six-month storage period, a minimum of 45% germination rate was observed in the seeds collected earliest during seed development (13 DAA) (Figure 3B); the percentage of dormant seeds decreased linearly thereafter (Figure 3C).

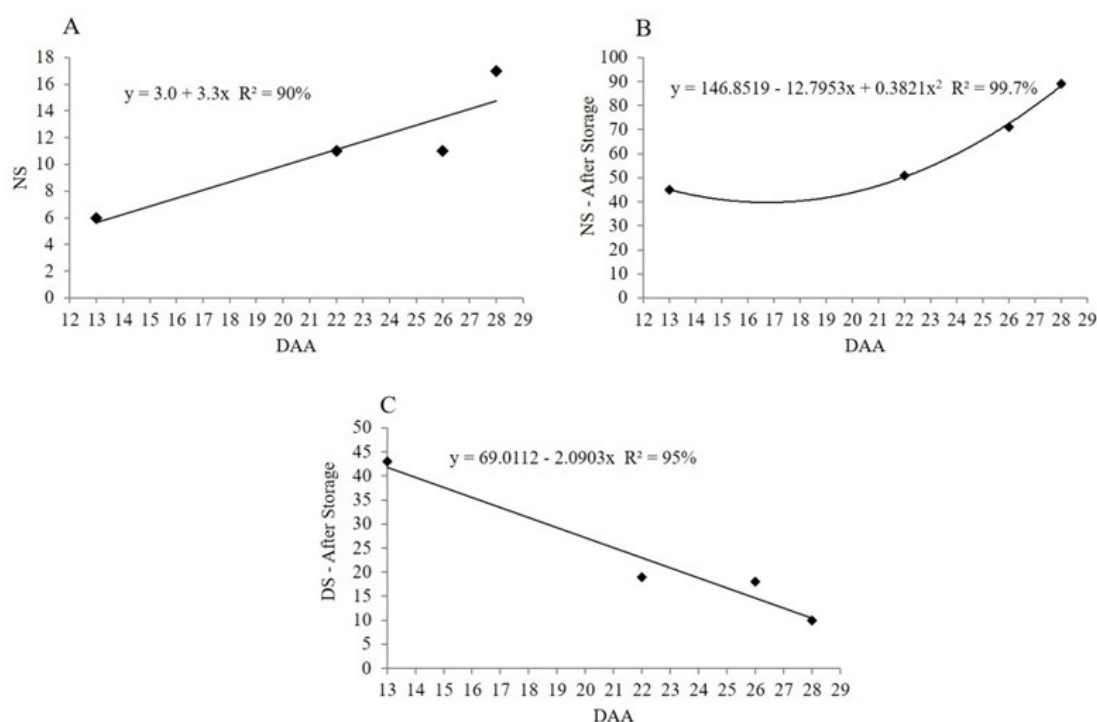


Figure 3. A – normal seedlings (%) derived from crambe seeds collected during different developmental stages; B - normal seedlings (%) derived from crambe seeds collected during different developmental stages, which remained in storage for a period of 180 days; C – dormant seeds (%) of crambe seeds collected during different developmental stages and stored for 180 days. DAA – days after anthesis.

It is worth noting that Oliveira et al. (2014) observed 76% germination in crambe seeds collected at 14 days after anthesis and immediately dehydrated at 10% water content under natural conditions, indicating the water loss may have accelerated the seeds to overcome deep dormancy. Dormancy is imposed by the combination of specific environmental conditions, which interfere with one or more blocking mechanisms, preventing the transcription of the genetic message for activation of the metabolic sequence that culminates in germination (Marcos Filho, 2015), i.e., ABI4 is mainly expressed during late seed maturation and appears to regulate after-ripening, a period of dry storage that releases dormancy (Shu et al., 2013).

Bessa et al. (2015) observed that in their natural environment, crambe seeds overcome dormancy within six months and showed a germination percentage within the commercialization standards for seeds in Brazil (80%, at least). Similar results were found in the present study after 180 days-storage, with increase in the

seeds germination collected at different developmental stages (Figure 3B) and concomitantly reduced the percentage of dormant seeds (Figure 3C).

Crambe seeds overcome dormancy during the storage period, while; at maturity they were still dormant. Field testing verified that the seeds were no longer dormant after the storage period and 71% of emergence was recorded (data not shown). It is worth mentioning that, even though seed dormancy was overcome after the storage period, seeds harvested 16 DAA and subsequently stored showed only 40% germination (Figure 3B), indicating that in the early stages of development, the seeds were still immature, and, thus, entered storage with reduced physiological quality.

According to the results, crambe seeds continued to develop until fully mature while being dormant, even though during maturation there was an inverse relationship between germinability and the presence of dormancy (Figure 3A), suggesting that the seeds acquire the capacity for germination during their development, but due to external and/or internal factors they developed while still in the dormant state. External factors are the climatic conditions to which the seeds were exposed during development, even though crambe is a rustic crop and well adapted to winter. At the same time, a reduction in water content was observed during seed development; until they reached maturity (Figure 2D), the seeds showed high water content, which can be associated with the presence of dormancy, indicating the need for a post-maturation period for the reduction of the water content, and subsequent germination. Bewley and Black (1994) observed that certain internal properties of the seeds can prevent germination, and, particularly in the developing seeds, is directly related to a high level of abscisic acid (ABA), which plays a role in preventing germination until natural dehydration of the seed occurred.

The process of seed post-maturation takes place when mature and recently-harvested seeds become non-dormant, that is, when they acquire the capacity for germination after a prolonged storage period (Bazin, Batlla, Dussert, El-Maarouf-Bouteau, & Bailly, 2011), indicating the presence of non-deep physiological dormancy in the crambe seeds (Bewley & Black, 1994). The need for a post-maturation process in seeds is common in many species and is usually associated with environmental conditions, such as the availability of oxygen, light, and temperature, which can promote seed germination, while, water content can also alter the rate at which seed dormancy is surpassed (Probert, 2000, Bazin et al., 2011, El-Maarouf-Bouteau et al., 2015).

The effect of the post-maturation period determined by seed storage under controlled conditions of temperature and relative humidity was fundamental to overcome dormancy and promote crambe seed germination. Even seeds that were harvested early during development (13 DAA) showed 45% germination after the storage period, suggesting influence on the mechanisms for overcome seed dormancy. Oliveira et al. (2014) observed an elevated percentage of germination (76%) in crambe seeds collected 14 DAA and subsequently dried to a water content of 10% that is, before reaching physiological maturity; thus, drying of the immature seeds was shown to help overcome dormancy. In contrast, crambe seeds harvested early during development, and which were not dried, showed an absolute requirement for a post-maturation process to overcome dormancy.

Possibly, seed dormancy at the end of seed maturation, when seeds still show a high water content, is part of a strategy to prevent the seeds from germinating while they are still attached to the mother-plant, and, thus, guarantee the survival of the species over winter. Orthodox seeds, such as crambe, can undergo several physiological modifications during storage—even while in a quiescent state—such as overcoming dormancy and losing viability. However, the mechanisms governing these processes have not been fully elucidated (Serhal, Leymarie, & Bailly, 2016).

According to the water-absorption curves of the first seeds harvest, the same absorption pattern was observed in all the seeds over the first 50 hours of soaking, i.e., rapid initial water absorption (Figure 4). After 50 hours of soaking, the hydration rates slowed down for the seeds harvested on all evaluation dates, thus signaling the start of the Phase II of the germination process (Figure 4), possibly characterized by the initiation of metabolic activities that convert endosperm reserve materials into the compounds required for germination. However, embryo growth did not resume until after ~300 hours of soaking, characterized by the stability observed in Phase II (Figure 4).

The freshly-harvested seeds showed an identical water-absorption behavior during the first 50 hours of soaking, such as rapid initial water absorption, characterized as Phase I of the triphasic pattern proposed by Bewley and Black (1994) (Figure 4), although the tendency curve shows a growing pattern. This phase is characterized by the rapid transfer of water from the growth substrate to the seed, due to the marked difference in water potential between them, with the entry of water taking place down an energy gradient that controls a series of dynamic biochemical events (Marcos Filho, 2015, Macovei et al., 2017). Such

behavior occurs both, in the non-dormant and dormant seeds, as observed in the crambe seeds at different stages of development (Figure 4).

There is an elevated water retention force in dehydrated seeds, but as the material becomes hydrated, these molecules occupy positions further and further away from the surface of the matrix, thus, reducing the retention force (Marcos Filho, 2015). The prevalence of dormancy in freshly-harvested crambe seeds explains the absence of Phase III, as there was no resumption of growth; instead, the stabilization of Phase II was verified until approximately 300 hours of soaking (Figure 4).

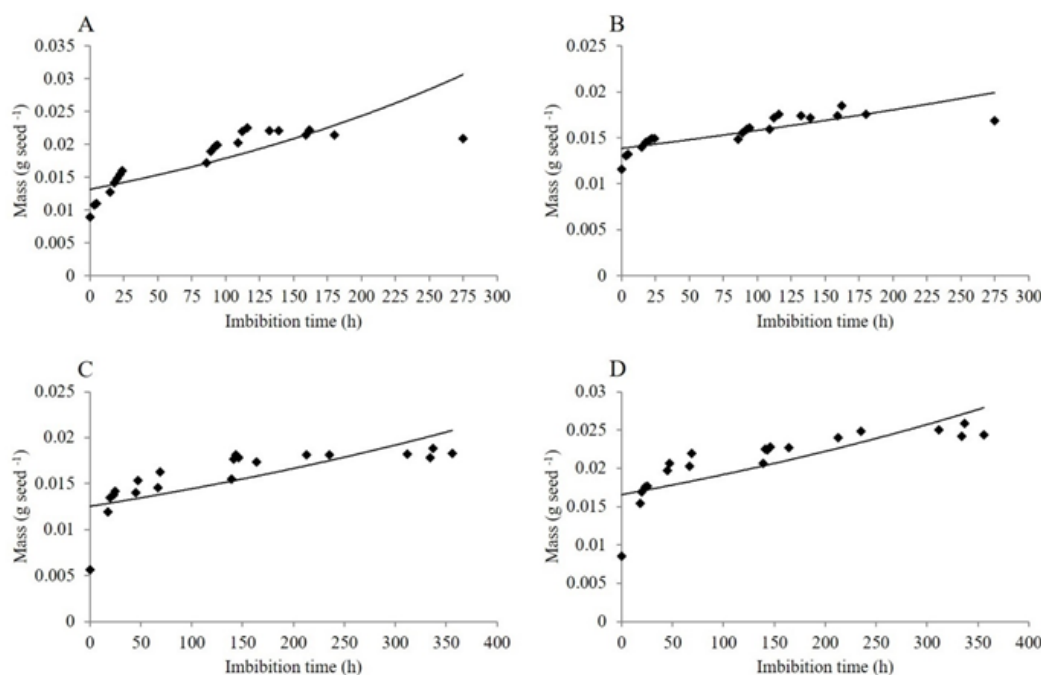


Figure 4. Water-absorption curves of crambe seeds collected (A) 13, (B) 22, (C) 26, and (D) 28 DAA (days after anthesis).

Our results suggest that the characterization of physical attributes, such as dry matter mass, size, and water content, may be more efficient parameters to identify maturity in crambe seeds, compared with physiological characteristics, such as germination, particularly due to the phenomenon of seed dormancy still observable at maturity. Another important contribution of the present study is establishing technology to evaluate the real germination potential; from our findings it was possible to understand the low efficacy assessment the crambe seeds germination soon after harvest; it was revealed a post-maturation period to overcome seed dormancy. In this sense, the evaluation of the physiological attributes of seed quality in isolation may negatively affect the determination of the point of seed maturity, especially, in crambe seeds, dispersed from the mother plant but still in a deep physiological state of dormancy.

Conclusion

Crambe seeds attain mature physical aspects with 29.5% water content at 28 days after flowering, but it is not accomplished by high germination levels, even if maximum seed dry matter is reached. During this period seeds were found in a deep state of dormancy and require a period post-maturation of 180-day storage to overcome seed dormancy.

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