Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

# Plant growth regulators and mobilization of reserves in imbibition phases of yellow passion fruit

## Reguladores vegetais e mobilização de reservas em fases de embebição de maracujá-amarelo

P. R. Manhone[a], J. C. Lopes[a], R. S. Alexandre[b], P. A. M. Lima[a], S. O. Lopes[a], L. H. G. Mengarda[a] and T. Mello[b]*

[a]Universidade Federal do Espírito Santo – UFES, Centro de Ciências Agrárias e Engenharias, Departamento de Agronomia, Alegre, ES, Brasil.

[b]Universidade Federal do Espírito Santo – UFES, Centro de Ciências Agrárias e Engenharias, Departamento de Ciências Florestais e da Madeira, Jerônimo Monteiro, ES, Brasil.

**Abstract**

The production of seedlings of the passion fruit tree, usually, is sexual, and the seeds are not uniform in the seedling emergence, and soaking treatments of seeds can provide faster and more uniform germination. It was aimed to study the action of plant growth regulators and the mobilization of reserves in the stages of soaking of yellow passion fruit seeds. The seeds were soaked for five hours in solutions containing plant growth regulators, in a completely randomized design, in a factorial 8 x 4, with four replications. The first factor corresponds to eight plant growth regulators: T1 - distilled water (control); T2 - 6-benzylaminepurine 500 mg $L^{-1}$; T3 - 4-(3-indolyl) butyric acid 500 mg $L^{-1}$; T4 - gibberellic acid 500 mg $L^{-1}$; T5 - spermine 250 mg $L^{-1}$; T6 - spermine 750 mg $L^{-1}$; T7 - spermidine 750 mg $L^{-1}$; T8 - spermidine 1250 mg $L^{-1}$; and the second factor, to the four soaking times: zero, four, 72 and 120 hours, corresponding, respectively, to the dry seed, and to phases I, II, and III of the imbibition curve. It was evaluated the biochemical composition of seeds (lipids, soluble sugars and starch). The seeds showed accumulation of lipids in phase III; the content of soluble sugars increased in phase I and decreased in phase II. The starch content increased until the phase II and decreased in phase III. Starch is the main reserve in the seeds and the main source of energy used in phase III; soaking the seeds in polyamines generates an accumulation of lipids in the seeds and soaking in plant growth regulators increases the burning of starch.

**Keywords:** *Passiflora edulis* L, lipids, starch, soluble sugars.

**Resumo**

A produção de mudas de maracujazeiro, geralmente, é sexuada, e as sementes não são uniformes na emergência das plântulas, e tratamentos de embebição das sementes podem proporcionar uma germinação mais rápida e uniforme. Objetivou-se estudar a ação de reguladores vegetais e a mobilização de reservas nas etapas de embebição de sementes de maracujá amarelo. As sementes foram embebidas por cinco horas em soluções contendo reguladores vegetais, em delineamento inteiramente casualizado, em esquema fatorial 8 X 4, com quatro repetições. O primeiro fator correspondeu a oito reguladores vegetais: T1 - água destilada (testemunha); T2 - 6-benzilaminapurina 500 mg $L^{-1}$; T3 - ácido 4-(3-indolil)butírico 500 mg $L^{-1}$; T4 - ácido giberélico 500 mg $L^{-1}$; T5 - espermina 250 mg $L^{-1}$; T6 - espermina 750 mg $L^{-1}$; T7 - espermidina 750 mg $L^{-1}$; T8 - espermidina 1250 mg $L^{-1}$; e o segundo fator, aos quatro tempos de embebição: zero, quatro, 72 e 120 horas, correspondendo, respectivamente, à semente seca, e às fases I, II e III da curva de embebição. Foi avaliada a composição bioquímica das sementes (lipídeos, açúcares solúveis e amido). As sementes apresentaram acúmulo de lipídeos na fase III; o teor de açúcares solúveis aumentou na fase I e diminuiu na fase II. O teor de amido aumentou até a fase II e diminuiu na fase III. O amido é a principal reserva nas sementes e a principal fonte de energia utilizada na fase III; a embebição das sementes em poliaminas gera um acúmulo de lipídios nas sementes e a embebição em reguladores de crescimento vegetal aumenta a queima do amido.

**Palavras-chave:** *Passiflora edulis* L, lipídios, amido, açúcares solúveis.

## 1. Introduction

The increase in demand for the consumption of fresh fruit and processed juice drives the expansion of the passion fruit cultivated area in Brazil, which already stands out as the world's largest producer of passion fruit, with an average production of ~ 690,364 tons of fruit in over 46 thousand hectares$^{-1}$ (IBGE, 2020). The fruit generates income in the field of US$ 4.8 billion a year (G1, 2021).

The passion fruit plants are propagated mainly through sexual reproduction with this, the selection of mother plants that provide seeds and the quality thereof is directly related to obtaining vigorous and disease-free seedlings.

*e-mail: tamyrisdemello@gmail.com
Received: May 9, 2023 – Accepted: January 16, 2024

However, the passion fruit seeds have problems related to their physiological quality, such as non-uniformity in the seedling emergence, which directly undermines the formation of seedlings, and soaking treatments of seeds can make them germinate faster and more evenly (Negreiros et al., 2006), as noted by (Marostega et al., 2015), wherein seeds immersion in distilled water at 50 °C for 5 minutes determined better germination percentage for *P. suberosa* species (34.69%).

The use of plant growth regulators on seed germination improves the seedling performance, accelerating the emergence speed and enhancing the vigor of the seeds of various species (Araujo Neto et al., 2018). The growth regulators polyamines are widely distributed throughout plant cells and exert a regulatory role on its growth and development, at micromolar concentrations. They are essential for vegetative growth, particularly for cell division and morphology (Chen et al., 2019). Auxins act on root formation and cell elongation, as they are involved in incorporating materials in the cell wall, working in the growth control of stem, leaves, roots and apical dominance. Gibberellins are involved in the synthesis of germination-specific protein and RNA, in both dormancy overcome and in controlling the hydrolysis of reserves, upon which the growing embryo depends (Taiz et al., 2017). The cytokinin is an important promoter of germination for being involved in the genes control, in the translation, in the regulation of protein functions and the regulation of membrane permeability (Farooq et al., 2022).

Osmotic adjustment is a physiological mechanism of plants that is activated in response to conditions adverse to its metabolism. It is effective in maintaining cell turgor, under conditions of low water potential in the soil and provides protection for short periods of time of stress, when conditions soon return to normal (Pintó-Marijuan and Munné-Bosch, 2013).

Thus, the importance of the imbibition curve is related both to tegument permeability studies and to the determination of the absorption period in seeds treated with plant growth regulators, osmotic priming and pre-hydration of seeds and increased seed respiratory activities at a level able to sustain the embryo growth, with sufficient supply of energy and organic substances, depending on the increase in the degree of hydration of its tissues (Carvalho and Nakagawa, 2012).

In general, during the period of seed formation, occurs, initially, an accumulation of sugars such as sucrose, fructose and glucose, and nitrogenous compounds such as amino acids and amides. These substances, which are drained from the mother plant, are critical for the formation of seed tissues and reserve substances that will be accumulated for the provision of energy and basic substances for starting the germination process. Thus, as the seed development occurs, there is a decrease in the number of these simpler substances and, at the same time, an accumulation of larger and more complex molecules such as proteins, starch, lipids and cellulose (Shibata et al., 2020).

The main reserve compounds in a seed are carbohydrates, lipids and proteins, altering in proportion in different species. The study of the chemical composition of a seed is also of practical interest in seed technology, because both the ability of seeds to express their vigor and the seeds storage period are influenced by the reserves contained therein (Taiz et al., 2017). At the beginning of the germination process, the reserves are mobilized, and during seedling development, its degradation products are used for different purposes, like power generation and the production of raw materials for the formation of cells and tissues (Calvi et al., 2017).

The use of starch or of soluble sugars varies depending on species, it may be during the germination or the seedling stage. In work done with soybeans (Henning et al., 2010), found that the seeds with greater vigor had higher content of soluble proteins, starch and soluble sugars, and higher mobilization capacity of reserves in the germination, resulting in soybean seedlings with better initial performance. Suda and Giorgini (2000) observed accumulation of soluble sugars in the embryo of *Euphorbia heterophylla* L. (wild poinsettia) during germination and found reduction in lipid content in the embryo after 72 and 96 hours of imbibition. These observations highlight the importance of reserve lipids in the embryonic axis in the early stages of germination.

Given that little is known about the reserves mobilization process in passion fruit seeds, the aim of this research was to know the action of plant growth regulators and the mobilization of reserves in the stages of soaking of the yellow passion fruit (*Passiflora edulis* Sims f. *flavicarpa* Deg.) seeds.

## 2. Materials and Methods

### *2.1. Plant material*

The experiment was conducted at the Seed Analysis Laboratory of Agricultural Sciences Center of the Federal University of Espírito Santo, located in the municipality of Alegre – ES.

Yellow passion fruit seeds were used, obtained from ripe fruits of several arrays forming a single batch. The extraction of seeds was carried out by fermentation for a period of 72 hours. After fermentation, the seeds were rinsed in sieve with current water and put to dry, for 24 hours, on paper towel on laboratory conditions (28 ± 2 °C), when it was carried out the determination of the moisture content of the lot (Brasil, 2009).

### *2.2. Treatment with plant regulators and statistical analysis*

The seeds were soaked for a period of five hours in solutions containing plant growth regulators. It was used a completely randomized design, in a 8 x 4 factorial arrangement (plant growth regulators and soaking times), with four repetitions of 25 seeds. The first factor corresponds to eight plant growth regulators, constituting the treatments: T1 - distilled water (control); T2 - 6-benzylaminepurine 500 mg $L^{-1}$ (6-BAP); T3 - 4-(3-indolyl) butyric acid 500 mg $L^{-1}$ (IBA); T4 - gibberellic acid 500 mg $L^{-1}$ ($GA_3$); T5 - spermine 250 mg $L^{-1}$ (ESM 250); T6 - spermine 750 mg $L^{-1}$ (ESM 750); T7 - spermidine, 750 mg $L^{-1}$ (ESD 750); T8 - spermidine, 1250 mg $L^{-1}$ (ESD 1250); and the second factor relates to the four soaking times of seeds: zero, four, 72 and 120 hours, corresponding, respectively, to the dry seed, and to phases I, II, and III of the soaking curve.

The three-phase pattern of water absorption by seeds is divided into phases I, II, and III, determined by Bewley and Black (1978). Phase I is defined as the phase of water absorption by the seed, and occurs in any material, even if the seed is dead. In this phase, the main events will be respiration and accumulation of ATP, activation of polysomes, mRNA synthesis and DNA repair, and protein synthesis from newly synthesized mRNA.

Phase II is defined by the interval of preparation and metabolic activation, in this stage water absorption reaches a plateau level, and the main events will be DNA synthesis and duplication, the beginning of cell elongation, the beginning of degradation of reserves, ending in protrusion of the primary root, a point that characterizes the beginning of phase III. This phase is defined by the growth of the embryo and is characterized by the visible beginning of germination, elongation followed by cell division, intense mobilization of reserves, formation of new molecules of carbohydrates, proteins, and lipids, and intense respiration of the seeds. In phase III, only live and non-dormant seeds complete the three-phase pattern.

Therefore, to determine the imbibition curve and define the times in which each phase occurs, the seeds were distributed in Petri dishes and soaked with distilled water to 50% of the contact surface and the substrate used was the germitest-type paper. The plates were kept in BOD germinator chambers at a temperature of 25 °C, in the absence of light. For the evaluation of water gain by seeds were carried out weighing every hour for the first eight hours, and after this period, the evaluations were performed every 24 hours up to 168 hours.

### *2.3. Quantification of lipids, starch and soluble sugars of seeds*

To quantify the reserves of seeds, four samples of 100 mg of dry seeds of each treatment were weighed. The samples were macerated with aid of crucible and pestle and placed in an Eppendorf tube containing 500 µL of chloroform and 1,000 µL of methanol, under constant stirring for 10 minutes and, then, it was added 500 µL of chloroform, stirring for further 10 minutes. The material was carried to the centrifuge for 5 minutes at 4,000 rpm. After this procedure, it was collected 650 µL of the supernatant, added 1,000 µL of distilled water, in an Eppendorf tube and the samples were homogenized with the aid of vortex mixing. After this period, there was the formation of two phases: the upper phase (methanol + water), whose solution was collected, quantitated and used for quantification of total soluble sugars; and the lower phase (chloroform), used to quantify the lipids, which were expressed in percentage.

To read the total soluble sugars, 15 µL aliquots of the upper phase (methanol + water) and 1,000 µL of anthrone were removed, placed in Eppendorf tubes and kept in bain-marie at 95 °C, for 15 minutes. Subsequently, the system was cooled to room temperature and reading the soluble sugars was performed with a spectrophotometer (Femto-cirrus 80 st), at 620 nm (Yemm and Willis, 1954), using four replicates per treatment.

The precipitate formed during the centrifugation in the the first Eppendorf tube was hydrolyzed with more than 1,000 µl of 3% HCl, for three hours, in bain-marie at 90 °C, in order to break down the starch. Hence, the starch was hydrolyzed to glucose, 10 µl aliquots were taken, another 1,000 µl of anthrone were added, and the reading for the total soluble sugars was performed.

The solution of the lower phase (chloroform + lipids) was maintained in 2 mL Eppendorf tubes identified and weighed. Then, they were put to dry in oven at 60 °C, and weighed again to quantify the resulting residue, consisting mostly of lipids (Bligh and Dyer, 1959).

## 3. Results

The initial moisture content of the seeds was 8%. (Figure 1). The phase with increased speed of water absorption occurred in the first 24 hours, where the seeds showed a 33% increase in water content (Figure 1); particularly in the first four hours of soaking, increasing 20%, while the phase of slower uptake occurred after 24 to 72 hours of imbibition, in which was observed a reduction in water uptake by seeds, from 41.32 to 41.83% water. Returning to absorb more water after 96 hours of soaking, rising water content to 45%, which marked the beginning of phase III. Based on the absorption curve, it was established the exact time of the three stages of soaking of yellow passion fruit seeds, the first 24 hours representing phase I; from 24 to 72 hours, phase II; and from 72 hours onwards, phase III.

The seeds, still dry, had the same lipid content (Figure 2). During phase I, the seeds soaked in treatment with polyamines (T8) had a higher percentage of lipids in relation to other treatments. The other treatments showed no change in the amount of lipid when compared. Although there was no difference in lipid content between treatments, seeds soaked in polyamines (T5, T6, T7 and T8) and cytokinin (T2) showed greater accumulation of this metabolite (phase II and III).

During phase II, the percentage of lipid found in the seeds soaked in gibberellin (T4) was lower due to the consumption of lipids by seeds. In phase II or lag phase, in which the digestion and active transport of reserve substances begins, in this phase there was almost no water absorption, initiating a stationary phase, in which there was an increase in the lipid concentration, except for the treatment with gibberellin (T4).

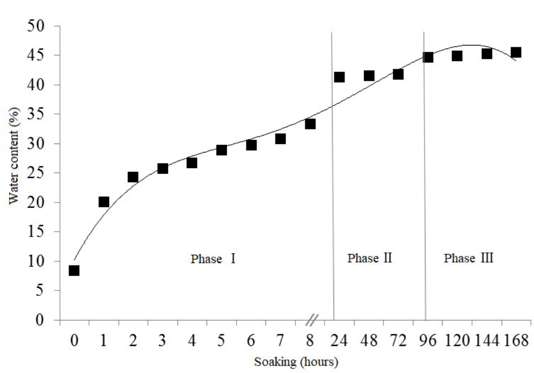


**Figure 1.** Curve of soaking seeds of yellow passion fruit (*Passiflora edulis* f. *flavicarpa* Sims Deg.).

During germination (phase III), the seeds soaked in the treatments with plant growth regulators showed a significant increase in the lipid content in relation to the control. The imbibition of seeds in spermidine (T8) showed more lipids after the first and second phases of imbibition (onset of germination).

In the quantification of the soluble sugar content in the first hours of imbibition (phase I), the passion fruit seeds showed increased soluble sugar content in all treatments, except in the treatment with distilled water (T1) (Figure 3). During phase II, in all treatments, the seeds showed decrease in the soluble sugar content, with the exception of the treatment made with lower concentration of the polyamine spermidine (T7). The sugar content during phase III markedly reduced in all treatments, behavior that may be associated with the primary root protrusion by seeds. During phase III, there was a lower mobilization of lipids in seeds, coinciding with the use of soluble sugar and the decrease in starch content during the same phase.

The starch content increased in all treatments during phases I and II of the imbibition and decreased after the beginning of the primary root protrusion (phase III) (Figure 4), except for the seeds soaked only in distilled water (T1), in which the starch levels continued to rise. Despite the low content of starch in the yellow passion fruit seeds, it seems to be important in the process of germination of these seeds, especially in seeds treated with gibberellin (T4) and polyamines (T5, T6, T7 and T8), since these had greater reduction in starch content in the transition from phase II to phase III (Figure 4).

The seeds soaked in a solution of polyamine spermidine (T7 and T8) showed greater decrease in the percentage of starch in relation to the quantity in the pre-protrusion period (phase II), 62 and 64%, respectively. The seeds treated with auxin (T3) had lower consumption of starch content compared to other treatments during the period of primary root protrusion (phase III).

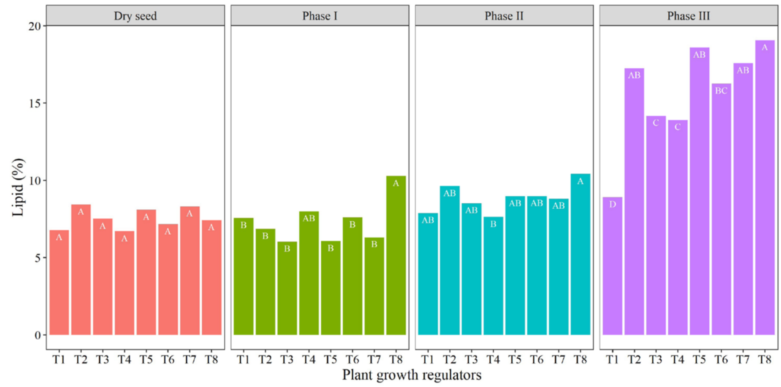


**Figure 2.** Lipid content during the soaking of the passion fruit seeds-yellow (*Passiflora edulis* Sims. f. *flavicarpa* Deg.) treated with plant regulators. Means followed by the same letter in the same color do not differ by Tukey's test at the 5% probability level. Legend: T1 - distilled water (control); T2 - 6-benzylaminepurine 500 mg $L^{-1}$ (6-BAP); T3 - 4-(3-indolyl) butyric acid 500 mg $L^{-1}$ (IBA); T4 - gibberellic acid 500 mg $L^{-1}$ ($GA_3$); T5 - spermine 250 mg $L^{-1}$ (ESM 250); T6 - spermine 750 mg $L^{-1}$ (ESM 750); T7 - spermidine, 750 mg $L^{-1}$ (ESD 750); T8 - spermidine, 1250 mg $L^{-1}$ (ESD 1250).

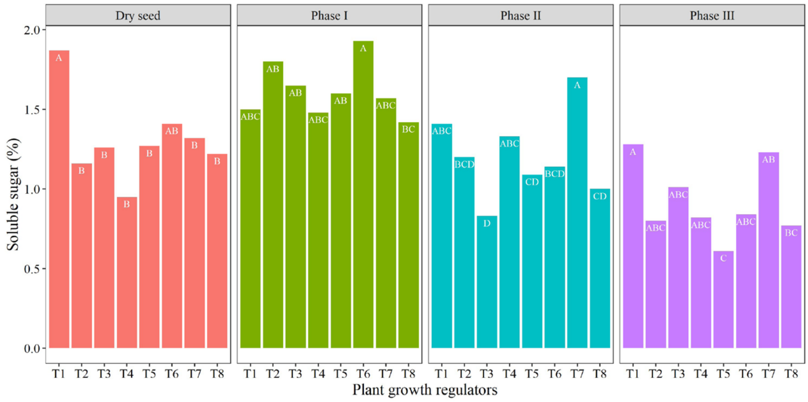


**Figure 3.** Soluble sugar content during the soaking of the passion fruit seeds-yellow (*Passiflora edulis* Sims. f. *flavicarpa* Deg.) treated with plant regulators. Means followed by the same letter in the same color do not differ by Tukey's test at the 5% probability level. Legend: T1 - distilled water (control); T2 - 6-benzylaminepurine 500 mg $L^{-1}$ (6-BAP); T3 - 4-(3-indolyl) butyric acid 500 mg $L^{-1}$ (IBA); T4 - gibberellic acid 500 mg $L^{-1}$ ($GA_3$); T5 - spermine 250 mg $L^{-1}$ (ESM 250); T6 - spermine 750 mg $L^{-1}$ (ESM 750); T7 - spermidine, 750 mg $L^{-1}$ (ESD 750); T8 - spermidine, 1250 mg $L^{-1}$ (ESD 1250).

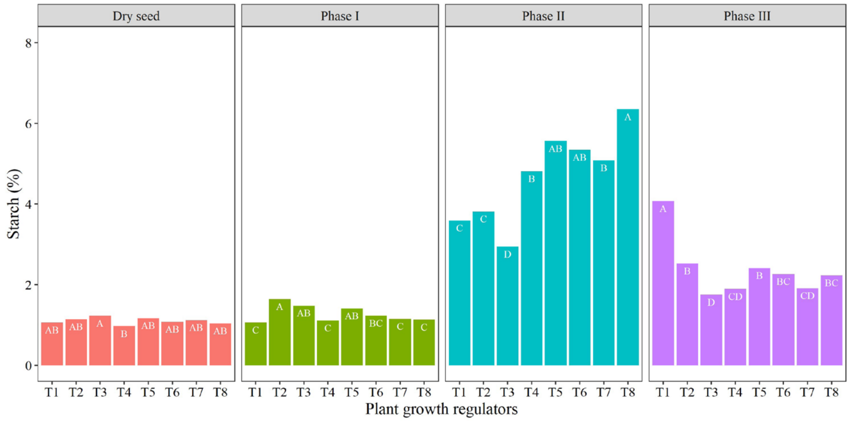


**Figure 4.** Starch content during the soaking of the passion fruit seeds-yellow (*Passiflora edulis* Sims. f. *flavicarpa* Deg.) treated with plant regulators. Means followed by the same letter in the same color do not differ by Tukey's test at the 5% probability level. Legend: T1 - distilled water (control); T2 - 6-benzylaminepurine 500 mg $L^{-1}$ (6-BAP); T3 - 4-(3-indolyl) butyric acid 500 mg $L^{-1}$ (IBA); T4 - gibberellic acid 500 mg $L^{-1}$ ($GA_3$); T5 - spermine 250 mg $L^{-1}$ (ESM 250); T6 - spermine 750 mg $L^{-1}$ (ESM 750); T7 - spermidine, 750 mg $L^{-1}$ (ESD 750); T8 - spermidine, 1250 mg $L^{-1}$ (ESD 1250).

## 4. Discussions

The absorption of water by the yellow passion fruit seeds during soaking, referring to the first two phases, followed the classic triphasic pattern of water absorption proposed by Bewley et al. (2013). The phase of slower uptake it is in this phase that occur the metabolic activities required for the protrusion of the primary root and the seedling development. And, in phase III occurred absorption associated with the initiation of the embryo growth, culminating in the protrusion of primary root, which means the end of germination (Mattana et al., 2018).

The spermidine, during phase I, may have accelerated, or advanced, the enzymatic process that started in phase II. The first soaking stage constitutes an essentially physical phenomenon which occurs due to the water potential difference between the seed and the environment (Bewley et al., 2013), a consequence of the matricial forces of the cell walls and of the cell content of the dry seeds, which can reach values up to -100 MPa; this increase in the water content occurs even if the seed is dormant, except when it shows imperviousness of the integument, ie, it is not feasible.

The accumulation of lipids in phases II and III may contribute to the energy supply for the growth and development of the embryo, showing reflections in the early establishment of the seedlings. While gibberellins may have a stimulating effect on the germination process and the seeds need this regulator to a series of events, like the activation of the vegetative growth of the embryo, the mobilization of endosperm reserves, and the weakening of the endosperm layer surrounding the embryo, favoring, thus, its growth (Taiz et al., 2017).

During phase II, the water potential of the environment and of the seed are very close and, with it, the water absorption by seed stabilizes. In this period, the activation of the pre-germinative metabolic processes occurs, since enzymes, membranes, and organelles such as mitochondria become active in the imbibed cells for the seeds to complete germination (Ali and Elozeiri, 2017). Assessing the *Caesalpinia peltophoroides* (sibipiruna) seed behavior (Corte et al., 2006), observed strong decrease in the amount of lipids from the beginning of soaking until the tenth day, being smoother from that moment onwards. Decrease even faster was found by [15], in seeds of *Euphorbia heterophylla* (wild poinsettia), whose degradation of lipids started soon after the initial seed soaking, being completed between 72 and 96 hours. On *Cucumis sativus* L. (cucumber), the degradation of lipids started only on the second day after germination, leaving 3% of the initial amount on the sixth day after germination.

The result of this research corroborates those obtained by Tozzi and Takaki (2011) by stating that lipids constitute the main source of energy for the germination of the yellow passion fruit seed. According to these authors, the yellow passion fruit seeds are classified as oilseeds, in virtue of its endosperm, rich in lipids. Souza et al. (2009), evaluating seeds of jatropha (*Jatropha curcas* L.), radish (*Raphanus sativus* L.) and crambe (*Crambe abyssinica* Hochst), also found higher lipid content in relation to sugars and starch. Lopes et al. (2013) also observed a significant increase (43%) in the lipid content in the period of protrusion of the primary root in jatropha seeds.

The amount of lipid found during the three phases of soaking in yellow passion fruit seeds follows the pattern observed in oilseeds, in which the lipid content remains unaltered during the soaking period, and decreases from the phase III (Ataíde et al., 2012). However, the lipid consumption does not follow a standard, since *Euphorbia heterophylla* seeds showed a decrease in lipid levels around 70% between three and four days after imbibition (Suda and Giorgini, 2000).

In the embryo development stage, the synthesis of total soluble sugars occurs. These are important in many physiological processes, acting as a source of energy, carbon skeletons, and/or as markers, being also insdispensable for the embryos to make them metabolically quiescent and tolerant to desiccation (Baud et al., 2002).

 

The reduction in sugar levels observed during the radicle pre-protrusion period (phases I and II) may be related to the activation of the initial seed metabolism, providing energy for germination before the initial mobilization processes of reserves could occur. The embryo uses as a source of energy, and substrate at cellular level, reserve and soluble sugars in the seed germination process.

It was verified, by Lopes et al. (2013), decrease in soluble sugar content from the early hours of soaking until the beginning of primary root protrusion in *Jatropha curcas* seeds. Pontes et al. (2002), working with seeds of *Apuleia leiocarpa* (Vogel) J.F. Macbr. (grápia), observed that the average content of soluble sugars did not differ significantly during the soaking period, nonetheless, there was a tendency to mobilize these reserves during this period.

Magalhães et al. (2010) found that the levels of total soluble carbohydrates in seeds of *Schizolobium parahyba* (Vell.) Blake (guapuruvu) showed marked reduction during the initial phase of soaking, and that this energy consumption is related to the respiration of seeds soon in the beginning of the imbibition process. To et al. (2002) found that in *Arabidopsis thaliana* (L.) Heynh. (arabeta, Portugal), the presence of soluble sugars, in general stemmed from the starch metabolism, inhibited the mobilization of lipid reserves.

The starch contents of this study are in agreement with the literature, the main function of starch is to provide energy for seedling germination and growth, until they become autotrophic (Bakhshy et al., 2020).

According to Melloni et al. (2012), the spermidine applied to leguminous seeds acts on the membrane integrity enabling the maintenance of the osmotic balance and the continuity of imbibition of the seeds, with positive effects on germination. According to Kubis (2008), the spermidine applied in rice cultivars seeds also prevented the extravasation of electrolytes and/or amino acids during soaking, enabling better germination.

The α-amylase, hydrolytic enzyme, which is produced by the aleurone layer in response to the action of gibberellins, is released in the endosperm where it operates in the conversion of starch into sugars, used for embryo growth (Bertagnolli et al., 2004). This activity can be observed mainly in the seeds soaked in the treatment T7; since these showed higher accumulation of soluble sugar in phase III of imbibition as a result of a greater combustion in the amount of starch in the previous phase (phase II) of seed imbibition.

Santos and Buckeridge (2004) showed that the presence of auxin leads to increased enzyme activity in the reserve metabolism. These authors were based on pH changes induced by the auxin in the extracellular matrix to explain this phenomenon. Enzymes dismantling the xyloglucan (polymers of wall constituent sugars with reserve function in cotyledons) have maximal activity at acidic pH. As auxin activates the transport of protons to the region of the cell wall, it would also increase the degradation of reserves. Notwithstanding, auxin production is regulated by light (Taiz et al., 2017). In this context, it is emphasized that the light and the free sugars are particularly important in coordinating the mobilization of the reserves and, in the case of this experiment, the absence of light during the soaking of seeds may have caused inhibitory effect of auxins in the germination process of the seeds.

## 5. Conclusions

In phase I, there is an increase in lipid content only in seeds treated with spermidine 1250 mg $L^{-1}$, compared to the content initially present in the seeds. As for the content of soluble sugars, there was an increase in all treatments (except the control treatment, where there was a decrease), mainly in seeds treated with spermine 750 mg $L^{-1}$. While the starch content practically does not change from the beginning to the end of phase I.

In phase II, the lipid content does not show considerable changes, compared to phase I, and the content of seeds treated with spermidine 1250 mg $L^{-1}$ remains higher. While soluble sugars in all treatments fall, and the seeds treated with spermidine 750 mg $L^{-1}$ remain with the highest averages. Starch percentages increase considerably in all treatments, especially in seeds treated with spermidine 1250 mg $L^{-1}$.

In phase III, the percentages of lipids present in the seeds of all treatments concerning phase II increase drastically, especially in seeds treated with spermidine 1250 mg $L^{-1}$. The content of soluble sugars and starch falls in all treatments, with a smaller drop in seeds without treatment with growth regulators, indicating that when treating the seeds, they mobilize a greater amount of carbohydrates for seedling growth.

Therefore, it can be determined that among the treatments with plant regulators tested, the best one for soaking yellow passion fruit seeds, to improve germination and seedling formation, is the use of spermidine 1250 mg $L^{-1}$.

## Acknowledgements

The authors thank the Federal University of Espírito Santo for providing facilities and equipment available for research; the Coordination for the Improvement of Higher Education Personnel (CAPES); the National Council for Scientific and Technological Development (CNPq) for financial support and research productivity grants and, respectively; to the Foundation for Research and Innovation Support of Espírito Santo (FAPES), for the granting research fee to the first author (FAPES Notice No. 19/2018 - Research Rate - FAPES Process Nº. 82195510).

## References

ALI, A.S. and ELOZEIRI, A.A., 2017. Metabolic processes during seed germination. In: J.C. JIMENEZ-LOPEZ, ed. *Seed biology*. London: IntechOpen, pp. 141-166. http://dx.doi.org/10.5772/intechopen.70653.

ARAÚJO NETO, A.C., SANTOS, O.O., FERREIRA, D.M., NUNES, R.T.C., PÚBLIO, A.P.P.B. and AMARAL, C.L.F., 2018. Germination and vigor of *Bixa orellana* L. seeds pre-soaked in a plant biostimulant. *Floresta*, vol. 48, no. 3, pp. 293-302. http://dx.doi.org/10.5380/rf.v48i3.43934.

ATAÍDE, G.M., FLORES, A.V. and BORGES, E.E.L., 2012. Physiological and biochemical changes in Pterogyne nitens tull. Seeds during the artificial aging process. *Pesquisa Agropecuária Tropical*, vol. 42, no. 1, pp. 71-76. http://dx.doi.org/10.1590/S1983-40632012000100010.

BAKHSHY, E., ZARINKAMAR, F. and NAZARI, M., 2020. Structural and quantitative changes of starch in seed of *Trigonella persica* during germination. *International Journal of Biological Macromolecules*, vol. 164, pp. 1284-1293. http://dx.doi.org/10.1016/j.ijbiomac.2020.07.262. PMid:32755696.

BAUD, S., BOUTIN, J.P., MIQUEL, M., LEPINIEC, L. and ROCHA, T.C., 2002. An integrated overview of seed development in *Arabidopsis thaliana* ecotype WS. *Plant Physiology and Biochemistry*, vol. 40, no. 2, pp. 151-160. http://dx.doi.org/10.1016/S0981-9428(01)01350-X.

BERTAGNOLLI, C.M., CUNHA, C.S.M., MENEZES, S.M., MORAES, D.M., LOPES, N.F. and ABREU, C.M., 2004. Qualidade fisiológica e composição química de sementes de soja submetidas ao estresse salino. *Revista Brasileira Agrociência*, vol. 10, no. 3, pp. 287-291.

BEWLEY, J.D. and BLACK, M., 1978. *Physiology and biochemistry of seeds in relation to germination*. Berlin: Springer, 306 p. http://dx.doi.org/10.1007/978-3-642-66668-1.

BEWLEY, J.D., BRADFORD, K.J., HILROST, H.W.M. and NONOGAKI, H., 2013. *Seeds: physiology of development, germination and dormancy*. 3rd ed. New York: Springer, 392 p.

BLIGH, E.G. and DYER, W.J., 1959. A rapid method for total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, vol. 37, no. 8, pp. 911-917. http://dx.doi.org/10.1139/o59-099. PMid:13671378.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária, 2009. *Regras para análise de sementes*. Brasília: MAPA/ ACS.

CALVI, G.P., AUD, F.F., FERRAZ, I.D.K., PRITCHARD, H.W. and KRANNER, I., 2017. Analyses of several seed viability markers in individual recalcitrant seeds of *Eugenia stipitata* McVaugh with totipotent germination. *Plant Biology*, vol. 19, no. 1, pp. 6-13. http://dx.doi.org/10.1111/plb.12466. PMid:27094237.

CARVALHO, N.M. and NAKAGAWA, J., 2012. *Sementes: ciências, tecnologia e produção*. 4. ed. Jaboticabal: FUNEP, 590 p.

CHEN, D., SHAO, Q., YIN, L., YOUNIS, A. and ZHENG, B., 2019. Polyamine function in plants: metabolism, regulation on development, and roles in abiotic stress responses. *Frontiers in Plant Science*, vol. 9, pp. 1945. http://dx.doi.org/10.3389/fpls.2018.01945. PMid:30687350.

CORTE, V.B., BORGES, E.E.L., PONTES, C.A., LEITE, I.T.A., VENTRELLA, M.C. and MATHIAS, A.A., 2006. Mobilization of the reserves during germination of seeds and growth of seedlings of *Caesalpinia peltophoroides* Benth. (Leguminosae-Caesalpinoideae). *Revista Árvore*, vol. 30, no. 6, pp. 941-949. http://dx.doi.org/10.1590/S0100-67622006000600009.

FAROOQ, M.A., MA, W., SHEN, S. and GU, A., 2022. Underlying biochemical and molecular mechanisms for seed germination. *International Journal of Molecular Sciences*, vol. 23, no. 15, pp. 8502. http://dx.doi.org/10.3390/ijms23158502. PMid:35955637.

G1, 2021 [viewed 7 June 2022]. *Agro: a indústria-riqueza do Brasil. Brasil é o maior produtor de maracujá do mundo*. Available from: https://g1.globo.com/economia/agronegocios/agro-a-industria-riqueza-do-brasil/noticia/2021/07/01/brasil-e-o-maior-produtor-de-maracuja-do-mundo.ghtml

HENNING, F.A., MERTZ, L.M., JACOB JUNIOR, E.A., MACHADO, R.D., FISS, G. and ZIMMER, P.D., 2010. Chemical composition and reserve mobilization in soybean seeds with high and low vigor. *Bragantia*, vol. 69, no. 3, pp. 727-734. http://dx.doi.org/10.1590/S0006-87052010000300026.

INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA – IBGE, 2020 [viewed 23 November 2021]. *Produção Agrícola Municipal 2020*. Available from: https://sidra.ibge.gov.br/tabela/5457.

KUBIŚ, J., 2008. Exogenous spermidine differentially alters activities of some scavenging system enzymes, $H_2O_2$ and superoxide radical levels in water-stressed cucumber leaves. *Journal of Plant Physiology*, vol. 165, no. 4, pp. 397-406. http://dx.doi.org/10.1016/j.jplph.2007.02.005. PMid:17658660.

LOPES, L.S., GALLÃO, M.I. and BERTINI, C.H.C.M., 2013. Mobilization of reserves during germination of Jatropha seeds. *Revista Ciência Agronômica*, vol. 44, no. 2, pp. 371-378. http://dx.doi.org/10.1590/S1806-66902013000200021.

MAGALHÃES, S.R., BORGES, E.E.L. and BERGER, A.P.A., 2010. Seed reserve mobilization in the embryonic axis and cotyledons of *Schizolobium parahyba* (vell.) S.F. Blake during germination. *Ciência Florestal*, vol. 20, no. 4, pp. 589-595. http://dx.doi.org/10.5902/198050982417.

MAROSTEGA, T.N., CUIABANO, M.N., RANZANI, R.E., LUZ, P.B. and SOBRINHO, S.P., 2015. Efeito de tratamento térmico na superação de dormência de sementes de *Passiflora suberosa* L. *Bioscience Journal*, vol. 31, no. 2, pp. 445-450. http://dx.doi.org/10.14393/BJ-v31n2a2015-22385.

MATTANA, E., SACANDE, M.,BRADAMANTE, G., GOMEZ-BARREIRO, P., SANOGO, S. and ULIAN, T., 2018. Understanding biological and ecological factors affecting seed germination of the multipurpose tree *Anogeissus leiocarpa*. *Plant Biology*, vol. 20, no. 3, pp. 602-609. http://dx.doi.org/10.1111/plb.12702.

MELLONI, M.L.G., CRUZ, F.J.R., SANTOS, D.M.M., SOUZA, L.F.G., SILVA, J., SACCINI, V.A.V. and MONTEIRO, J.G., 2012. Espermidina exógena atenua os efeitos do NaCl na germinação e crescimento inicial de leguminosas forrageiras. *Revista Brasileira de Sementes*, vol. 34, no. 3, pp. 495-503. http://dx.doi.org/10.1590/S0101-31222012000300018.

NEGREIROS, J.R.S., WAGNER JÚNIOR, A., ALVARES, V.S., SILVA, J.O.C., NUNES, E.S., ALEXANDRE, R.S., PIMENTEL, L.D. and BRUCKNER, C.H., 2006. Influência do estádio de maturação e do armazenamento pós-colheita na germinação e desenvolvimento inicial do maracujazeiro amarelo. *Revista Brasileira de Fruticultura*, vol. 2, no. 1, pp. 21-24. http://dx.doi.org/10.1590/S0100-29452006000100009.

PINTÓ-MARIJUAN, M.P. and MUNNÉ-BOSCH, S, 2013. Ecophysiology of invasive plants: osmotic adjustment and antioxidants. *Trends in Plant Science*, vol. 18, no. 12, pp. 660-666. http://dx.doi.org/10.1016/j.tplants.2013.08.006. PMid:24001766.

PONTES, C.A., BORGES, E.E.L., BORGES, R.C.G. and SOARES, C.P.B., 2002. Mobilização de reservas em sementes de *Apuleia leiocarpa* (Vogel) J.F. Macbr. (garapa) durante a embebição. *Revista Árvore*, vol. 26, no. 5, pp. 593-601. http://dx.doi.org/10.1590/S0100-67622002000500010.

SANTOS, H.P. and BUCKERIDGE, M.S., 2004. The role of the storage carbon of cotyledons in the establishment of seedlings of *Hymenaea courbaril* L. under different light conditions. *Annals of Botany*, vol. 94, no. 6, pp. 819-830. http://dx.doi.org/10.1093/aob/mch209. PMid:15514028.

SHIBATA, M., COELHO, C.M.M., STEINER, N., BLOCK, J.M. and MARASCHIN, M., 2020. Lipid, protein and carbohydrate during seed development in *Araucaria angustifólia*. *Cerne*, vol. 26, no. 2, pp. 301-309. http://dx.doi.org/10.1590/01047760202026022653.

SOUZA, A.D.V., FÁVARO, S.P., ÍTAVO, L.C.V. and ROSCOE, R., 2009. Caracterização química de sementes e tortas de pinhão manso, nabo forrageiro e crambe. *Pesquisa Agropecuária Brasileira*, vol. 44, no. 10, pp. 1328-1335. http://dx.doi.org/10.1590/S0100-204X2009001000017.

SUDA, C.N.K. and GIORGINI, J.F., 2000. Seed reserve composition and mobilization during germination and initial seedling development of *Euphorbia heterophylla*. *Revista Brasileira de Fisiologia Vegetal*, vol. 12, no. 3, pp. 226-245. http://dx.doi.org/10.1590/S0103-31312000000300006.

TAIZ, L., ZEIGER, E., MOLLER, I. and MURPHY, A., 2017. *Plant physiology and development.* 6th ed. Porto Alegre: Artmed.

TO, J.P.C., REITER, W. and GIBSON, S.I., 2002. Mobilization of seed storage lipid by *Arabidopsis* seedlings is retarded in the presence of exogenous sugars. *BMC Plant Biology*, vol. 2, no. 4, pp. 4-15. http://dx.doi.org/10.1186/1471-2229-2-4. PMid:11996676.

TOZZI, H.H. and TAKAKI, M., 2011. Histhochemical analysis of seed reserve mobilization in *Passiflora edulis* Sims f. *flavicarpa* O. Deg. (yellow passion fruit) during germination. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 71, no. 3, pp. 701-708. http://dx.doi.org/10.1590/S1519-69842011000400015. PMid:21881794.

YEMM, E.W. and WILLIS, A.J., 1954. The estimation of carbohydrate in plant extracts by anthrone. *The Biochemical Journal*, vol. 57, no. 3, pp. 508-514. http://dx.doi.org/10.1042/bj0570508. PMid:13181867.