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Degradation of reserves of crambe seed (*Crambe abyssinica*) during germination

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The aim of this study was to determine how lipids, total soluble sugars and starch are degraded during the germination process of crambe seeds (FMS Brilhante Cultivar). The experiment consisted of collecting seeds during every day of the germination test (0, 1, 2, 3, 4, 5, 6 e 7 days) during which were also held the germination counts. After collection, the proportion of seeds and / or seedlings was designed for the determination of water content, and the other part was separated to perform biochemical analysis. The study adopted a completely randomized design with four replications and the means were compared by regression analysis. Crambe seed reserves showed degradation since the metabolic activation with increased germination in that lipid, soluble sugars, and starch are degraded.

Key words: Lipids, total soluble sugars, starch.

INTRODUCTION

The crambe (*Crambe abyssinica* Hochst) belonging to the Brassicaceae family, originated from the Mediterranean region with occurrence in Ethiopia, and being recently introduced in Brazil as an alternative to forage and off-season crop production (Colodetti et al., 2012). The plant has a relatively short cycle of 90 to 120 days, requiring approximately 52 days between sowing and flowering, considerable tolerance to water stress, soil and saline irrigation water and also resistance to low temperatures (Pitol et al., 2010) and productivity of 1428,98 kg ha^{-1} (Brandão et al., 2013). The industrial use of crambe oil is suggested to be a raw material for biodiesel production because their seeds have oil content of up to 38% (Pitol et al., 2010) and features such as kinematic viscosity, density and acid value are suitable for biodiesel production (Silva et al., 2013).

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The crambe oil extracted from the seed may be used as a lubricant in the manufacture of plastic films and in the drug composition. The crambe oil cannot be used for human consumption due to the presence of high erucic acid content, a long chain monounsaturated fatty acid, which causes damage to the heart when present in the human body (Colodetti et al., 2012).

In addition to the purely industrial point of view, it should be considered that the yield obtained in the industries is potentially dependent on the quality and yield of plants. Thus, the germination of this kind becomes important to any industrial process which is intrinsically associated with the mode degradation of its reserves. The main substance stored in crambe seeds are lipids, but carbohydrates and proteins are also found into smaller proportions (Oliva et al., 2012).

During germination, reservations are hydrolyzed and mobilized to embryo growth (Kucera et al., 2005). Lipids and carbohydrates are used as an energy source (Pritchard et al., 2002) and protein to provide amino acids for the formation of new tissues (Ramakrishna, 2007).

Plants need to convert the more lipids stored in a mobile form of carbon, often as sucrose, because they are not able to carry fats cotyledon to other tissues during development of the seedlings (Taiz and Zeiger, 2013). According to Graham (2008) oilseeds metabolize the triacylglycerols stored, converting them to sucrose following germination. The conversion of sucrose to lipid in oil seed germination is initiated by starting with the hydrolysis of stored triglycerides lipids as free fatty acids, and subsequent oxidation of these fatty acids to produce acetyl-CoA. Fatty acids are oxidized in glioxissomo, and acetyl-CoA is metabolized in glioxissomo and cytoplasm to produce succinate, which is transported to mitochondria and converted to fumarate and then malate, and cytosolic malate is converted to glucose via gluconeogenesis and then as sucrose (Taiz and Zeiger, 2013).

Considering the importance of the degradation of reserves during the germination of seeds, the aim of this study was to determine how lipids, total soluble sugars and starch are degraded during the germination process of crambe seeds.

MATERIALS AND METHODS

Installation, experimental design and data analysis

The seeds were purchased from the Mato Grosso do Sul Foundation. The experiment was conducted in the Germination, Seed Dormancy and Plant Physiology Laboratory II, of the Department of Botany, Biosciences Institute, UNESP – Botucatu / SP. The experiment consists of seeds collection during every day of the germination test (0, 1, 2, 3, 4, 5, 6 and 7 days) during which the germination counts were also held. Four replications of 50 seeds were placed for each treatment. After collection, the proportion of seeds and/or seedlings was designed for the determination of water content, and the other part was separated to perform biochemical analyzes.

A completely randomized design was adopted with four replications and the means were compared by regression analysis ($p \leq 0.05$).

Seed moisture content

The water content was determined by the oven method at 105 ± 3°C for 24 h, using three replicates of 4.5± 0.5 g. The results were expressed on a wet basis (Brasil, 2009).

Germination

The germination test was conducted with four replications of 50 seeds for each treatment. The seeds were placed on blotting paper, moistened in water for 2.5 times the mass of dry substrate, packed in transparent plastic boxes (11 x 11 x 3.5 cm). They were then placed on germination B.O.D. in alternating temperature and photoperiod (light - 30°C for 8 h and dark - 20°C for 16 h).

Total soluble sugars

The extraction of total soluble sugars was carried out according to the methodology of Garcia et al. (2006) with minor modifications. The seeds were pulverized with a pestle and mortar in N_2 liquid. To obtain the alcoholic extract, 100 mg of the pulverized seeds were homogenized in 1 mL of 80% ethanol (v/v) and incubated for 15 min at 80°C. The homogenate was centrifuged at 12,000 *g* for 15 min at room temperature. At the end of centrifugation, the supernatant was removed and reserved. This procedure was performed three times for the complete removal of total soluble sugars, combining the supernatants from the three extractions at the end. Then, the final volume was adjusted to 3 ml with deionized water resulting in the alcoholic extract. These extract was stored in separate microtubes at -20°C until determination. The pellets were stored at -20°C for further starch extraction.

For the determination of total soluble sugars, the methodologies used were taken from Morris (1948) and Yemm and Willis (1954). The anthrone reagent was prepared dissolving 0.1 g of anthrone in 45 mL of sulfuric acid 95% (v/v). The reaction mixture consisted of 50 µL of alcoholic extract + 950 µL deionized water (final volume 1000 µL), kept in an ice bath, and 2000 µL of cold anthrone solution was added. The reaction mixture was incubated for 3 min at 100°C. After cooling, the total sugar content was determined by taking the absorbance at 620 nm using glucose as a standard and was expressed as mg per mg of dry weight.

Starch

For extraction of starch, the study followed the the method described by Clegg (1956) with minor modifications. The pellet, derived from the alcoholic extract, was homogenized in 500 µL of deionized water, in an ice bath. To the homogenate was added 650 µL of 52% perchloric acid (v/v) and kept in an ice bath for 15 min (shaken every 5 min). Then, 2000µL of deionized water was added and centrifuged at 12,000 *g* for 15 min at 4°C.

At the end of centrifugation, the supernatant was removed and reserved. This procedure was performed again, however the mixture containing pellet + deionized water + 52% perchloric acid (v/v) was kept in an ice bath for 30 min (shaken every 5 min) and centrifuged at 12,000 *g* for 15 min at 4°C. The two supernatants were combined at the end. These extract was stored in separate microtubes at -20°C until determination of starch content. The determination of starch content was performed like the total sugar determination.

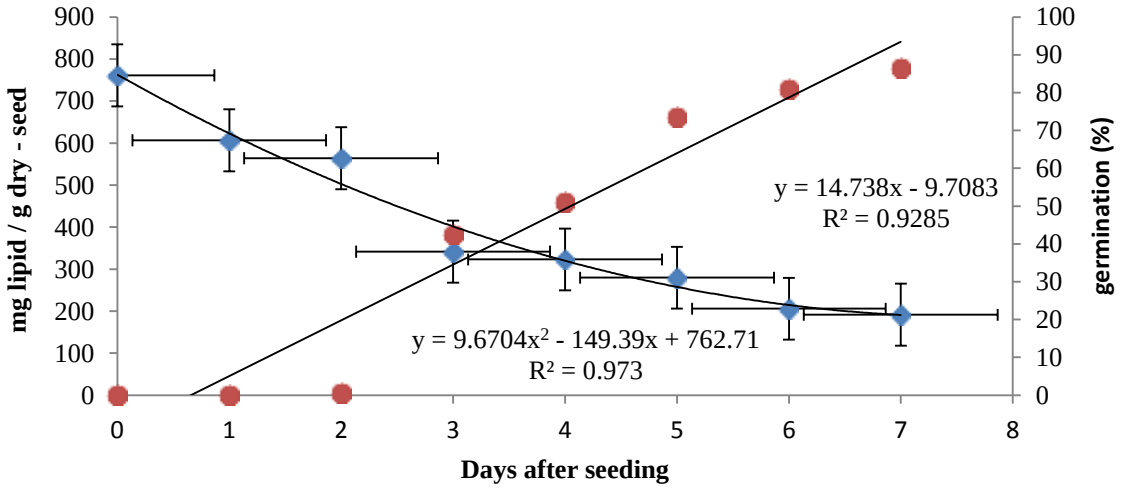

Figure 1. Degradation of lipids during germination crambe seeds (p≤0.05).

Total lipid

The quantification of total lipids was performed according to the method of Manirakiza et al. (2001) and Ambalkar et al. (2011). To obtain the extract, about 1.0 to 2.0 grams of sample soaked in liquid nitrogen was weighed. In these samples, 100 ml of hexane was added, arranged in flat-bottom flasks and placed in fatty material extract in 3 cycles of 8 h. Subsequently, the material was filter while hot, and brought to a rotary evaporator for separation of solvent and lipids. Lipids were removed from the flasks with the aid of a Pasteur pipette, transferred to glass jars with lid, and subsequently weighed.

RESULTS AND DISCUSSION

The results of germination of crambe seed, obtained during the 7-day evaluation and the degradation reservations are presented in tables. It can be seen that seed germination process started on the third day of evaluation.

The data relating to lipid content in the seeds set to a decreasing quadratic regression equation, indicating that there has been rapid consumption of reserves during the germination period from the first day after the metabolic activation (Figure 1).

In *Cucumis sativus* L. the degradation of lipids started on the 2nd day after germination, leaving only 3% of total initial six days. This rapid degradation begins with the emergence of the radicle and ends with the complete expansion of the cotyledons (Matsui et al., 1999). However, according Suda & Giorgini (2000) this pattern is unusual when compared to other oil seeds, in which the lipid content remains unchanged during the initial period of germination, diminishing. It was observed in *Arabidopsis thaliana* (L.) that the degradation of lipid reserves is inhibited in the presence of soluble sugars such as glucose and sucrose, and in general stemmed starch metabolism (To et al., 2002).

The total soluble sugar seeds also exhibit marked reduction during the initial stage of germination, during imbibition (Day 1), indicating their use in breathing, with subsequent stabilization of cotyledons between 3 and 6 days (Figure 2) and decrease in the seventh day. Borges et al. (2002) and Buckeridge and Dietrich (1996) verified the consumption of sucrose and raffinose during germination and *Sesbania marginata, Platymiscium pubescens*, respectively, considering the first two reservations of soluble sugars to be used. The data relating to starch content in the seed set to a downward quadratic regression equation indicates the intake and mobilization of reserves during the germination period (Figure 3). According to Magalhaes et al. (2010), starch provides glucose to be used both as air for breath, to generate electricity, and to compose physical structures for embryo growth during germination phase.

In summary, crambe seed germination lipids, soluble sugars and starch are degraded quickly providing energy for the development of the embryo during germination, which is completed at 7 days after metabolic activation, when observing the emission of the primary root. Thus, while lipid reserves are considered slow in degradation, crambe seed process starts from the moment the seeds are placed in contact with water and the metabolism is activated.

Conclusion

Crambe seed reserves had degradation since the metabolic activation with increased germination in that lipid, soluble sugars and starch are degraded.

Conflicts of interest

The authors have not declared any conflict of interest.

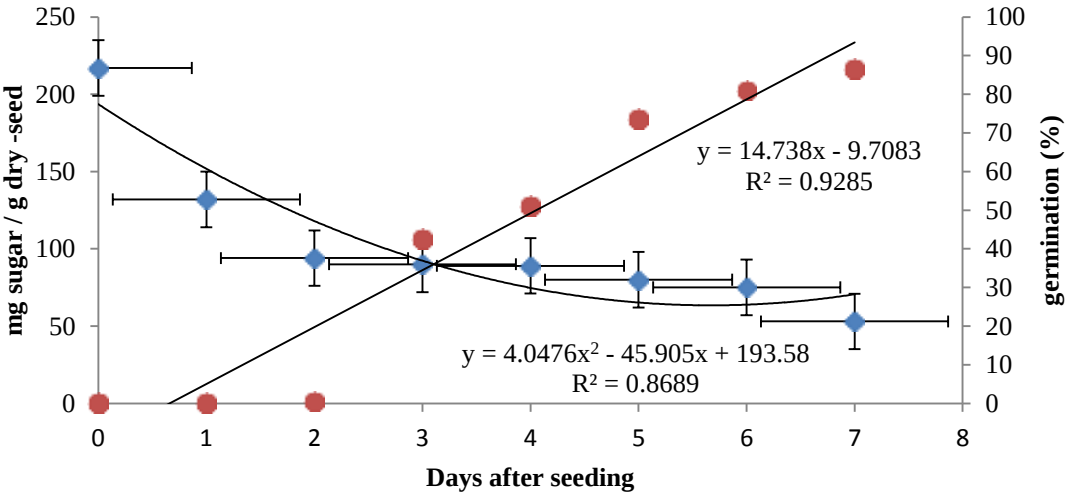

Figure 2. Degradation of total soluble sugar during germination crambe seeds (p≤0.05).

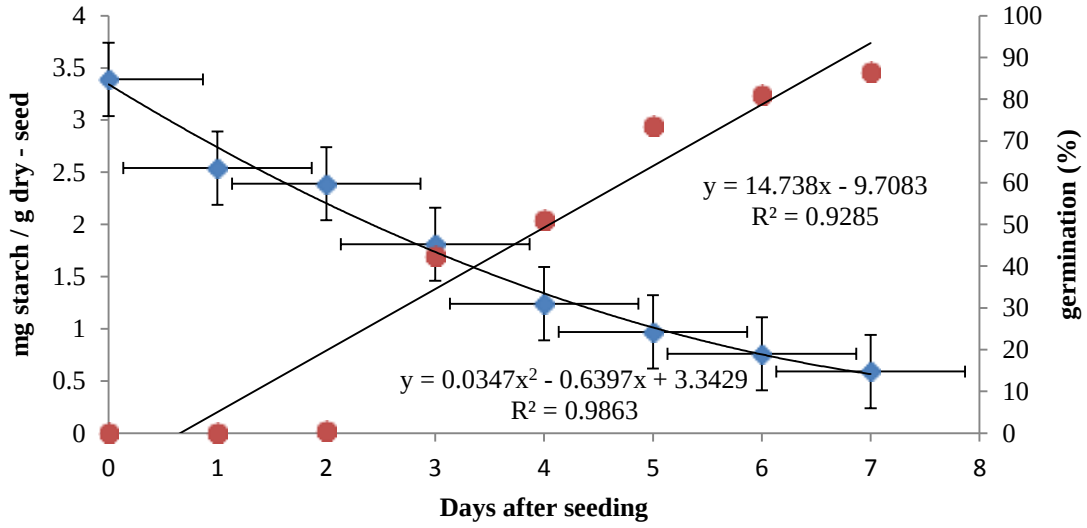

Figure 3. Degradation of starch during germination crambe seeds (p≤0.05).

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