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Research article

Hormonal profile and the role of cell expansion in the germination control of Cerrado biome palm seeds

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ABSTRACT

Little information is currently available concerning the mechanisms controlling palm seed germination. We compared the anatomical and physiological aspects of seeds of two neotropical palm species showing different levels of dormancy. The seeds of *Attalea vitrivir* and *Butia capitata* were evaluated for the endogenous contents of hormones (ABA, GAs, CKs, BRs, IAA, JA, SA and the ethylene precursor ACC) in their cotyledonary petiole and operculum (structures involved in germination control), the force necessary to displace the operculum, endo-β-mannanase activities, and embryo cell elongation. The analyses were carried out on with intact dry and imbibed seeds as well as with seeds with the operculum mechanically removed, 2, 5 and 10 days after sowing. The germinabilities of the intact seeds of *A. vitrivir* and *B. capitata* were 68% and 3%, respectively; the removal of the operculum increased germination to more than 90% in both species. Reductions of ABA and increases in GAs contents coincided with cell elongation, although there is no evidence that hormonal balance and endo-β-mannanase activity are involved in operculum weakening. The ratio between the embryo length and the force required for operculum displacement (EL/OF) was found to be 1.9 times greater in *A. vitrivir* than in *B. capitata*, which means that very small elongations in each cell would be sufficient to promote germination, resulting in a lower level of dormancy in the former species. EL/OF and cell growth control are therefore important for defining dormancy level in palm seeds.

1. Introduction

Dormancy is common in palm seeds, resulting in slow and uneven germination that may take years to complete (Orozco-Segovia et al., 2003). Germination in Arecaceae is complex due to the anatomical and physiological characteristics of their diaspores, and several types of dormancy have been reported (Orozco-Segovia et al., 2003; Baskin and Baskin, 2014). Although recent studies have provided information on the role of seminal structures and the physiological mechanisms involved in the control of germination in that family (Bicalho et al., 2015; Carvalho et al., 2015; Ribeiro et al., 2015), some issues deserve to be more thoroughly investigated.

Dormancy is overcome in most cases due to increased embryo growth and/or the weakening of adjacent tissues – balance-mediated processes involving primarily the hormones gibberellins (GAs) and abscisic acid (ABA), but also cytokinins (CKs), brassinosteroids (BRs) and ethylene (Kucera et al., 2005; Finch-Savage and Leubner-Metzger, 2006; Nambara et al., 2010). The hormonal control of germination in palm seeds has only been broadly investigated in *Elaeis guineensis* Jacq. (Jiménez et al., 2008) and *Acrocromia aculeata* (Jacq.) Lodd. ex Mart. (Bicalho et al., 2015; Ribeiro et al., 2015), and it is not clear how seed structures interact in these processes.

The seeds of Arecaceae have a structure called the operculum (composed of the opercular tegument and micropillary endosperm) that limits embryo growth (Hussey, 1958). The micropylar

Abbreviations: ABA, abscisic acid; ACC, 1-aminocyclopropane-1-carboxylic acid; BL, brassinolide; BRs, brassinoesteroids; CKs, cytokinins; CS, castasterone; CT, cathasterone; DHZ, dihydrozeatin; DHZR, dihydrozeatin riboside; GAs, gibberellins; IAA, indole 3-acetic acid; IPA, isopentenyladenosine; JA, jasmonic acid; SA, salicylic acid; Z, zeatin; ZR, zeatin riboside; 2-iP, 2-isopentenyladenine.

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endosperm contains mannan-rich cell walls (Carvalho et al., 2015), and hydrolase enzymes in the seeds of species of different plant families act to mobilize those compounds (endo-β-mannanase being one of the principal enzymes), promoting the weakening of tissues adjacent to the embryo (Buckeridge, 2010; Bewley et al., 2013). There are, however, doubts about the role of these enzymes in germination in the family Arecaceae (Gong et al., 2005).

Palm embryo cotyledons have two principal regions: the cotyledonary petiole and the haustorium. The embryonic axis has only microscopic dimensions and is contained within the cotyledonary petiole (DeMason, 1988; Orozco-Segovia et al., 2003). While the operculum and the cotyledonary petiole are directly involved in germination, the haustorium functions in the mobilization of the endosperm reserves (Ribeiro et al., 2015; Mazzottini-dos-Santos et al., 2016) (Fig. 1A, C). Unlike most plant species, the structure that first emerges and indicates the conclusion of the germination in palms is the cotyledonary petiole – not the primary root (Neves et al., 2013; Oliveira et al., 2013; Carvalho et al., 2015). These peculiarities make it difficult to evaluate germination and classify dormancy in the palm family, raising the need for more detailed studies on the processes of embryo growth during germination and its interactions with adjacent tissues.

Attalea vitrivir Zona and *Butia capitata* (Mart.) Becc. are economically important endemic species of the Cerrado biome (Neotropical savanna) – a region characterized by marked climatic seasonality. The fruits of *A. vitrivir* are used in the production of charcoal, and its oil-rich seeds have much promise for biodiesel production (Neves et al., 2013). *B. capitata* has ornamental potential, and the fruit pulp is currently used in the food industry for manufacturing juices, ice cream and liqueurs (Magalhães et al., 2013).

The seeds of *A. vitrivir* show lower dormancy levels (referred to here as indicative of the percentage of dormant seeds in a population, according to Bewley et al., 2013) than those of *B. capitata.* This apparently influences the distribution patterns of their populations (Carvalho et al., 2015), although there is no in-depth information available concerning the mechanisms determining those differences. In that context, we compared the anatomical and physiological aspects of the seeds of both species to define the role of cell expansion, its interaction with hormonal profile (20 hormones, belonging to eight classes) and endo-β-mannanase activity, and discuss here the mechanisms that control germination and the observed differences in dormancy levels among palm species.

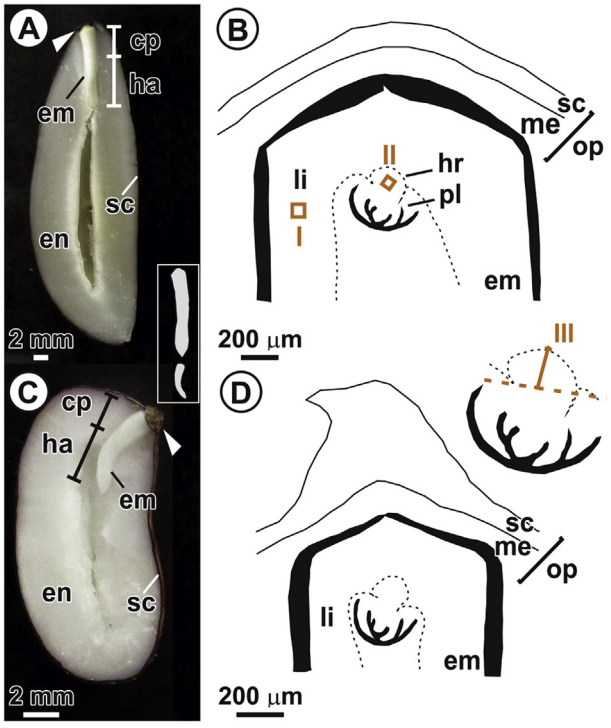

Fig. 1. Morphoanatomy of *Attalea vitrivir* (A, B) and *Butia capitata* (C, D) seeds. A,C, Longitudinally sectioned seeds with arrowheads indicating the operculum. B,D, views of the micropylar region, with dashed lines highlighting procambial strands. B, view indicating (orange) the measured regions: lengths of ligule cells (I), lengths of hypocotyl-radicle axis cells (II); and lengths of the hypocotyl-radicle axis (III). A scheme of the embryos drawn with the same scale used in A is presented on the right side of the images, to facilitate the comparison of the dimensions. em, embryo; cp, cotyledonary petiole; ha, haustorium; en, endosperm; hr, hypocotyl-radicle axis; li, ligule; me, micropylar endosperm; op, operculum; pl, plumule; sc, seed coat. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2. Methods

2.1. Fruit collection and preliminary procedures

Mature fruits of *A. vitrivir* were collected (after natural abscission) from a natural population in the municipality of Januária (15° 25′ 30.3″ S, 44° 40′ 49.8″ W); mature fruits of *B. capitata* were harvested from a natural population in the municipality of Bonito de Minas (15° 25′ 59.8″ S, 44° 41′ 31.7” W), both in the northern region of Minas Gerais State, Brazil. For both species, the samples were collected in a minimum of 20 individuals, in the harvests of 2013/2014. The seeds were extracted from the fruits (Dias et al., 2013; Neves et al., 2013) and kept for up to 30 days in a dry and aerated place (average 25 °C and 65% RH). Before the beginning of the experiments the seed water contents were determined by drying them at 105 °C (Brasil, 2009), using four replicates of 10 seeds each.

2.2. Evaluation of germination

After surface sterilization in 6% sodium hypochlorite for 15 min and three rinses with distilled water, the seeds were kept immersed in water under laboratory conditions for 5 days (with daily renewal of the water) (Neves et al., 2013; Oliveira et al., 2013). The operculum was removed from half of the imbibed seeds with the aid of scalpel, without damaging the embryo (Dias et al., 2013; Neves et al., 2013). Five replicates of 20 intact seeds and 20 seeds without their operculum were placed in polyethylene containers containing sterilized vermiculite (moistened to 80% of its retention capacity) and maintained at 30 °C for 30 days. The temperature of 30 °C was used because it is considered adequate for seed germination in *A. vitrivir* (Neves et al., 2013) and in most of tropical palms species (Orozco-Segovia et al., 2003). Although there are no published studies with tests of different temperatures for germination of *B. capitata* seeds, we considered as reference a study in which this temperature was used for both species (Carvalho et al., 2015) and recent results obtained by our research group (unpublished data). Germination was recorded daily (considered as the protrusion of the cotyledonary petiole) (Ribeiro et al., 2015), and the daily percentage germinations were calculated.

Dried seeds, imbibed seeds, and seeds sown to germinate (with and without their operculum) for two, five and 10 days were dissected to obtain cotyledonary petioles and operculum samples (in those treatments in which those structures were maintained) for physiological analyses (Fig. 2), those structures being excised using a razor blade. The cotyledonary petioles from the seeds of all treatments were used for anatomical evaluations. The water contents of the seeds in each treatment were determined (Brasil, 2009).