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ANATOMICAL ALTERATIONS IN MICROPYLAR ENDOSPERM OF *Melanoxylon brauna* SCHOTT. SEEDS DURING GERMINATION

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*ABSTRACT – **Melanoxylon brauna** Schott. is an endangered Brazilian species with potential use in reforestation programs. Owing to these reasons, a better understanding of its germination process is an important step towards its preservation. Our study aimed to evaluate the anatomical and enzymatic changes in the micropylar endosperm of **Melanoxylon brauna** seeds during germination. Seeds were germinated at 25 ºC. Samples for evaluations of anatomy was soaked at 25ºC for 0, 16, 24, 48 and 72h. During the first 72h of soaking, a reduction of micropillary endosperm thickness and consumption of lateral endosperm cells was observed. suggesting these water soaking is an important role in the enzymes activation to germination process.*

Keywords: *Seeds metabolism; Histochemistry; Native species.*

1. INTRODUCTION

Melanoxylon brauna Schott. (Brauna) is a native species from the Atlantic Forest (Brazil), occurring in the states of Bahia, São Paulo, Minas Gerais, Espírito Santo, Pará, Rio de Janeiro and Sergipe. Due to the exploitation of its wood, this species is currently in the "Official list of Brazilian flora species threatened with extinction", in the vulnerable category, according to the Brazilian Ministry of Environment. Therefore, information on the physiology and seed germination may contribute to the development of new strategies for the conservation of this species (MMA 2014; Ataíde et al. 2016).

Germination involves a number of relatively complex physiological and biochemical processes (Bewley et al. 2013). During the germination process, dynamic changes occur in embryonic cells involving reactive oxygen species (ROS), proteins and enzymes that play a role in the changes that take place in the cell wall. The lower resistance of the cell wall, a result from the activity of these enzymes, contributes to the cells' growth, which results in protrusion of the primary root (Zhang et al. 2014).

Little is known about the mechanisms related to the rupture of the micropyle region, especially the weakening of the tissues involved in this biological process (Wang et al. 2004; Zhang et al. 2014). However, it is accepted that the elongation of the hypocotyl radicle axis and the weakening of the tissues of the micropyle are involved in germination. These two processes work together to provide protrusion of the radicle and both of them require the loss of cell wall integrity through the actions of hydrolases, transglycosylases, cellulases, and hemicellulases such as: endobetamananase, alpha-galactosidase, polygalacturonase and pectinamethyl esterases (PME) (Nonogaki 2014; Wang et al. 2014; Zhang et al. 2014).

In order to understand the germination process, it is important to consider the anatomical and histochemical changes that occur from the beginning of imbibition until the moment of root protrusion. However, there are few studies that describe the anatomical, structural and histochemical changes that occur during germination and their relationship with physiological and biochemical changes.

Therefore, the aim of this study was to evaluate the anatomical changes in the micropylar *M. brauna* seeds during germination in order to determine whether the growth of embryo and the weakening of the tissues that surround it are mechanisms involved in *M. brauna* germination.

2. METHODOLOGIES

2.1. Anatomical changes

The *M. brauna* seeds imbibed at 25 ºC for 0, 16, 24, 48 and 72 h were separately fixed in FAA_{50}, (formaldehyde: acetic acid and ethyl alcohol (50%, 5: 5: 90, v / v / v) during 48 h and they were stored in 70% ethanol (Johasen 1940). Subsequently, the biological material was included in methacrylate (Historesin-Leica), according to the manufacturer's recommendations. The samples were longitudinally sectioned on a 5µm thick autoclave rotary microtome (model RM2155, Leica Microsystems Inc., Deerfield, USA) and stained with toluidine blue (O'brien et al. 1964). Then, permanent blades were prepared on synthetic resin (Permount®). The images for the anatomical analysis were obtained by light microscope (model AX-70 TRF, Olympus Optical, Tokyo, Japan) coupled with a digital photographic camera (Zeiss AxioCam HRc model, Göttinger, Germany) and microcomputer with the Axion Vision image capture program.

3. RESULTS

3.1. Anatomical Changes

Germination started after the first 72 h of imbibition, reaching an average of 83%. The *Brauna* seed consists of tegument, endosperm and embryo. It is, therefore, an albuminous seed, with hard tegument, dark brown in color and a dense endosperm. It also has a layer of macrosclereids, with the presence of Malpighi cells, which are cells with thick walls, strongly joined together, perpendicularly arranged in relation to the surface, constituting the exotesta. Differential thickness of this structure was observed, being higher in the micropyle region. Internal to the macrosclereids is the hypoderm, consisting of a layer of osteosclereids, which are sclerified, columnar and bulbous cells shaped like an hourglass (Fig. 1a).

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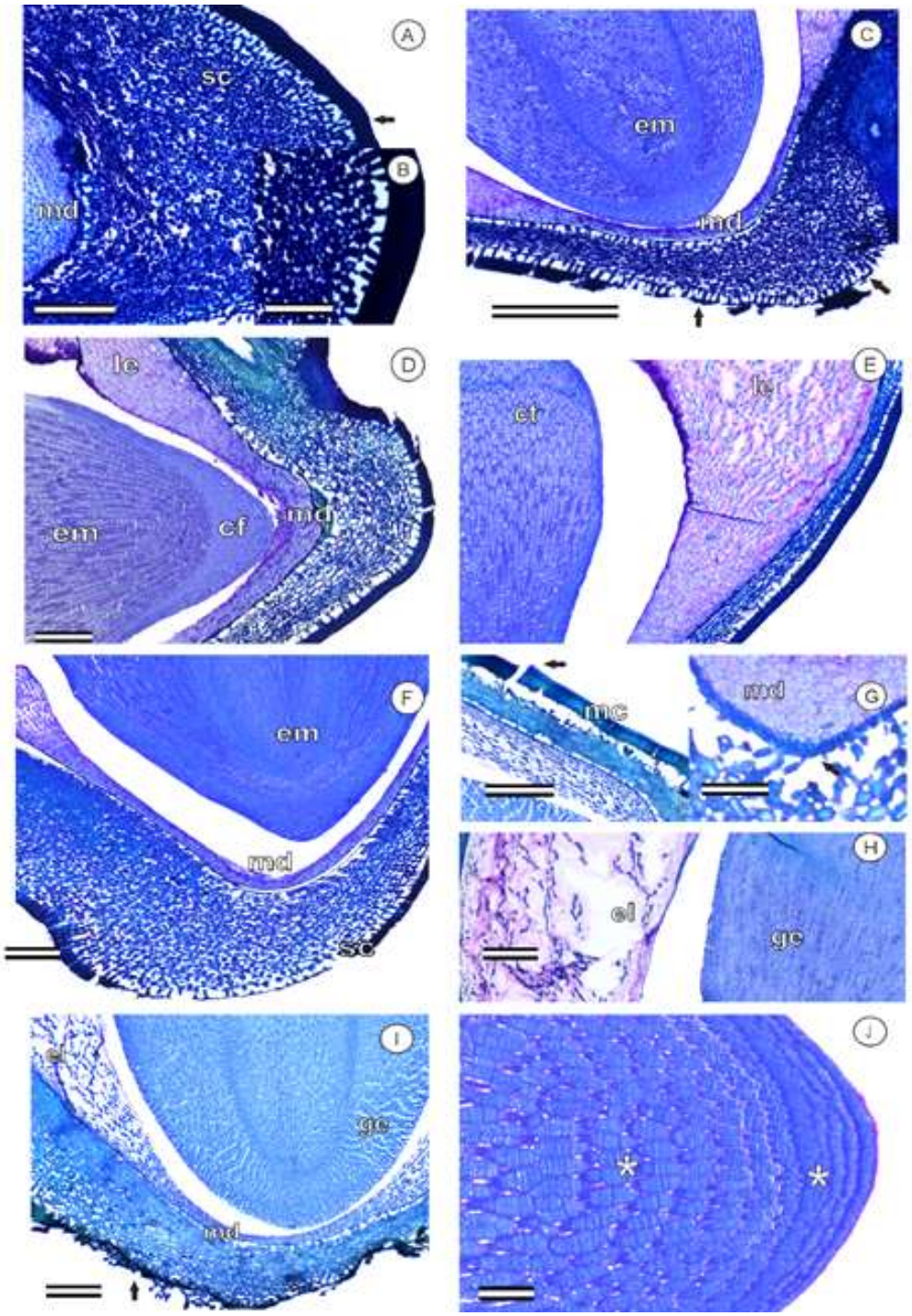

Figure 1 – Anatomical changes during the soaking process of *Melanoxylon brauna* seeds. a = control group (0 h soaking); b = detail of intact integument (0 h); c = 16 h; d = 24 h; e = detail of lateral endosperm consumption (24 h); f = detail of embryo preparing for germination (24 h); g = 48 h; h = lateral endosperm consumption (48 h); i = integument detrition (72 h) and j = detail of embryonic axis cells (72 h). Arrows indicate weakening of endosperm during germination. Asterisks indicate division and elongation of embryo cells. Em = embryo; le = lateral endosperm; mc = micropyle; md = endosperm. Bars = 300 µm; exception: B = 200 µm.

consisting of a layer of osteosclereids, which are sclerified, columnar and bulbous cells shaped like an hourglass (Fig. 1a). The imbibition caused a reduction in the thickness of the micropylar endosperm, the onset of consumption of the lateral endosperm cells and cracks in the integument (Fig. 1c). Additionally we identified cracks on the integument (Fig. 1d) and the formation of a protective region that will give rise to the root cap (Fig. 1d). After 24h of imbibition, the consumption of cell wall material (macro and osteosclereids) and lateral endosperm were observed (Fig. 1e). At the same stage of germination, we observed the development of the embryo with elongation of the hypocotyl radicle axis (Fig. 1f). After 48h of imbibition, the tissue that composed the outermost layers of macro and osteosclereids were almost completely consumed in the micropylar region (Fig. 1g). Whereas in the lateral regions, although less consumed, the presence of ruptures and cracks were observed (Fig. 1i) with evident wear on these tissues and on the more internal tissues, especially in the micropylar region. The lateral endosperm was almost completely consumed, wi th large voids (Fig. 1).

Anatomical changes during the soaking process of *Melanoxylon brauna* seeds. a = control group (0 h soaking); b = detail of intact integument (0 h); c = 16 h; d = 24 h; e = detail of lateral endosperm consumption (24 h); f = detail of embryo preparing for germination (24 h); g = 48 h; h = lateral endosperm consumption (48 h); i = integument detrition (72 h) and j = detail of embryonic axis cells (72 h). Arrows indicate weakening of endosperm during germination. Asterisks indicate division and elongation of embryo cells. Em = embryo; le = lateral endosperm; mc = micropyle; md = endosperm. Bars = 300 µm; exception: B = 200 µm.

4. DISCUSSION

The beginning of the germination process was observed before the first 24 h of imbibition, demonstrated by the tegumentary wear (Fig. 1c). The forces of potential growth of the embryo and the weakening of the tissue that limits it act concomitantly, contributing to the protrusion of the radicle through the tissues around it (Yan et al. 2014; Bewley et al. 2013).

The sequence of events described in Fig. 1 differs from the hypothesis described in the literature. This hypothesis indicates that the consumption of the micropylar endosperm precedes that of the lateral endosperm in order to facilitate root protrusion (Muller et al. 2013; Bewley et al. 2013; Yan et al. 2014). However, in *Brauna* seeds the consumption of the lateral endosperm begins before and after it occurs concomitantly to the consumption and wear of the micropylar endosperm.

In other species, the micropylar endosperm is the first to be consumed during imbibition, as in tomato, wheat, barley, rice, watercress and tobacco seeds (Lee et al. 2012; Yan et al. 2014). This difference between the sequence of events may be associated with several factors, such as tegument thickness, imbibition rate, morphoanatomic characteristics and phytohormone action.

Our results show that the embryo potential growth force and the weakening of tissues that act as a physical barrier are the two mechanisms with opposite forces that are involved in the germination of seeds with rigid seed coat, such as *M. brauna*. Alterations in tegument and consumption of the lateral endosperm begin within the first 24 h of imbibition, with onset of lateral endosperm and subsequent weakening of the micropylar endosperm. The weakening of the micropylar endosperm by the action of the enzymes, and water is essential for conclusion of germination by endosperm rupture.

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6. REFERENCES

Ataíde GM, Borges EEL, Gonçalves JFC, Guimarães VM, Flores AV (2016) Alterações fisiológicas durante a hidratação de sementes de Dalbergia nigra ((Vell.) Fr. All. ex Benth.). Ciênc Florest 26(2):615-62. doi:10.5[02/1980509822761

Bewley JD, Black M (2013) Seeds: physiology of

development and germination. Springer Science & Business Media

Borges EEL, Ataíde GM, Matos ACB (2015) Micropilar and embryonic events during hydration of *Melanoxylon brauna* Schott seeds. J. Seed Sci. 37(3): 192-201. doi: 10.1590/2317-1545v37n3147846

Brazilian Ministry of Environment – MMA (2014) Instrução Normativa nº43 de 18 de setembro de 2014. Lista Oficial das Espécies da Flora Brasileira Ameaçadas de Extinção. Portaria n. 443, December 17th, 2014. Diário Oficial da União, 18/12/2014, Seção 1, p. 110-121

Dixit S, Stein PK, Dewland TA, Dukes JW, Vittinghoff E, Heckbert SR, Marcus GM (2016) Consumption of caffeinated products and cardiac ectopy. J of the American Heart Association 5(1): 1-11. doi:10.1161/JAHA.115.002503

Jayany RS, Saxena S, Gupta R (2005) Microbial pectinolytic enzymes: A review. Process Biochemistry 40(9):2931–2944. doi: 10.1016/j.procbio.2005.03.026

Johasen DA (1940) Plant microtechnique. Mcgraw-Hill Book, New York.

Lee KJD, Dekkers BJW, Steinbrecher T, Walsh CT, Bacic A, Bentsink L, Leubner-Metzger G, Knox JP (2012) Distinct Cell Wall Architectures in Seed Endosperms in Representatives of the Brassicaceae and Solanaceae. Plant Physiol 160(3):1551-1566. doi:10.1104/pp.112.203661

Muller K, Levesque-Tremblay G, Bartels S, Weitbrecht K, Wormit A, Usadel B (2013) Demethylesterification of cell wall pectins in Arabidopsis plays a role in seed germination. Plant Physiol 161: 305–316. doi: 10.1104/pp.112.205724

Nonogaki H (2014) Seed dormancy and germination-emerging mechanism and new hypotheses. Front Plant Sci 5(233):1-14. doi: 10.3389/fpls.2014.00233

O'brient TP, McCully ME (1981) The study of plant structure: principles and selected methods. Termarcarphi Pty, Melbourne.

Schopfer P (2001) Hydroxyl radical-induced cell-wall loosening in vitro and in vivo implications for the control of elongation growth. Plant J 28(6):678-688. doi: 10.1046/j.1365-313x.2001.01187.x

Wang Q, Goldstein M, Alexander P, Wakeman TP, Sun T, Feng J, Lou Z, Kastan MB, Wang XF (2014) Rad17 recruits the MRE11-RAD50-NBS1 complex to regulate the cellular response to DNA double-strand breaks. Embo J 33(8):862-77. doi:10.1002/embj.201386064

Yan D, Duermeyer L, Leoveanu C, Nambara E (2014) The functions of the endosperm during seed germination. Plant Cell Physiol 55(9):1521-33. doi: 10.1093/pcp/pcu089

Zhang Y, Chen B, Xu Z, Shi Z, Chen S, Huang X, Chen J, Wang XF (2014) Involvement of reactive oxygen species in endosperm cap weakening and embryo elongation growth during lettuce seed germination. J of Experimental Botany 65(12): 3189-3200. doi: 10.1093/jxb/eru167