Alterations in seed reserves of *Dalbergia nigra* ((Vell.) Fr All. ex Benth.) during hydration[1]

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ABSTRACT – Seed imbibitions is the first stage of the germination process and is characterized by the hydration of tissues and cells and the activation and/or induction of the enzymes responsible for mobilizing reserves for respiration and the construction of new cell structures. The objective of this study was to investigate the alterations in reserve substances during slow hydration of Bahia Rosewood (*Dalbergia nigra*) seeds in water. Seeds from two different lots (Lot I and II) were placed in saturated desiccators (95-99% RH) to hydrate at 15 and 25 °C until water contents of 10, 15, 20 and 25% were reached. At each level of hydration, changes in lipid reserves, soluble carbohydrates, starch and soluble proteins were evaluated. The mobilization of reserves was similarly assessed in both lots, with no differences being observed between the two hydration temperatures. Lipid contents showed little variation during hydration, while the contents of soluble carbohydrates and starch decreased after the 15% water content level. Soluble proteins showed a gradual tendency to decrease between the control (dry seeds) up to 25% water content.

Index terms: carbohydrates, imbibition, Bahia rosewood, lipids, proteins.

Alterações nas reservas de sementes de *Dalbergia nigra* ((Vell.) Fr. All. ex Benth.) durante a hidratação

RESUMO - A embebição das sementes é o passo inicial do processo de germinação, que se caracteriza pela hidratação de tecidos e células, ativando e/ou induzindo a síntese de enzimas responsáveis pela mobilização das reservas, para atender as necessidades de respiração e a construção de novas estruturas celulares. Portanto, o objetivo deste trabalho foi investigar as alterações nas substâncias de reservas durante a hidratação lenta em água de sementes de jacarandá-da-bahia (*Dalbergia nigra*). Sementes pertencentes a dois lots distintos (lots I e II) foram colocadas para hidratar em dessecadores saturados (95-99% UR) nas temperaturas de 15 e 25 °C até atingirem os teores de água de 10, 15, 20 e 25% de umidade. Em cada nível de hidratação foram avaliadas as variações nas reservas de lipídios, carboidratos solúveis, amido e proteínas solúveis. A mobilização de reservas ocorreu de maneira similar nos dois lotes avaliados, porém não foram observadas diferenças entre as duas temperaturas de hidratação. Os teores de lipídios apresentaram pequena variação durante a hidratação, enquanto os conteúdos de carboidratos solúveis e amido apresentaram decréscimo a partir do nível de hidratação de 15%. Proteínas solúveis exibiram tendência gradativa de queda, desde a testemunha (sementes secas) até o nível de 25% de umidade.

Termos para indexação: carboidratos, embebição, jacarandá-da-bahia, lipídios, proteínas.

Introduction

The discussion about the need for propagating native species to reclaim and restore the natural landscape in the face of current environmental problems has been increasing in recent years, as has also been seen regarding seed production for food and industrial purposes. The generation of data on the germination physiology, cultivation and the potential of these species can provide important information for different programs of forest management, forestry plantations and forest enrichment.

However, more details on the germination process of the seeds of native species are necessary, since despite their

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ecological and environmental importance, there is little information on how best to propagate the native species originating from different biomes. In the Atlantic Forest, for example, species such as *Dalbergia nigra*, popularly known as Bahia Rosewood, jacarandá-caviúna, or black rosewood (Papilonoidea), is known for its moderately hard, heavy, decorative and naturally long-lasting wood, which gives this species a high commercial value (Rizzini, 1972; Lorenzi, 1992). Due to these commercial characteristics, this tree species has been heavily exploited and is on the list of plant species threatened with extinction, being classified as vulnerable (Ibama, 2008). On the other hand, little has been done to further scientific research on the propagation strategy(s), especially germination and the study of soaking, embryo growth and seedling establishment.

Germination usually starts with imbibition, when the metabolic and physiological processes associated with germination begin and the embryo grows (Nonogaki et al., 2007). In this case, water is the main agent in the germination process, affecting the percentage, the uniformity and speed of germination. Water absorption by the seed varies between species (Alvarado and Bradford, 2002) and seed hydration, or pre-hydration, is the gradual imbibition of water by seeds, under controlled conditions of temperature and relative humidity. This procedure allows a seed to activate many stages of the germination process, but not radicle protrusion (Varier et al., 2010). Therefore, seed water content may be an important factor affecting the water absorption and the subsequent germination response (Long et al., 2010).

During the germination process, changes occur in the chemical composition of the seed and the use of reserve substances, principally carbohydrates, lipids and proteins, which are hydrolyzed and provide energy for the synthesis of protoplasm and structural components and embryo development (Buckeridge et al., 2004; Kerbauy, 2004). The lipids are used as a source of carbon and energy for seedling development (Leonova et al., 2010), while the seed carbohydrates are used as a substrate for respiration during pre-germination, with starch serving as a source of reduced carbon for seed respiration and metabolism (Bewley and Black, 1994). The proteins are hydrolyzed to amino acids by proteolytic enzymes and provide a source for synthesizing new enzymes and structural proteins (Wang et al., 2007).

Considering, therefore, the importance of tropical native species from the ecological aspect and the potential use of their wood, it is essential to know in more detail the physiological and biochemical mechanisms of the distribution and behavior of the seeds under different environmental conditions, in particular, seed hydration. The objective of this study was to investigate changes in lipid, soluble carbohydrate, starch and soluble protein reserves during hydration of *Dalbergia nigra* seeds, in order to understand the behavior of these reserves during seed water absorption.

Materials and Methods

This study was conducted in the seed laboratory of the Department of Forest Engineering, Federal University of Viçosa (UFV), between September/2010 and July/2011. The fruits of *Dalbergia nigra* used for analysis were collected, when brown/greenish, direct from two trees of different origins in the region of Viçosa, Minas Gerais state. The fruits were processed and the seeds constituted lots I and II. During the processing, immature, deteriorated or damaged seeds were eliminated. The selected seeds were packed in fiber drums and stored in a cold chamber at 5 °C and 60% humidity until use.

The water content of the seeds of lots I and II was determined in an oven at 105 ± 3 °C, using three replicates of 20 seeds for each treatment, with the results expressed as a percentage (wet basis), according to methodology described by the Rules for Seed Testing (Brasil, 2009).

Samples from the two seed lots were germinated in Petri dishes on two sheets of germitest paper, moistened with distilled water under continuous light provided by four 40 W fluorescent, daylight-type lamps at a constant temperature of 25 °C for 12 days (Ferraz-Grande and Takaki, 2001). Seeds were considered germinated when the radicle protruded. The germination speed index (GSI) was calculated according to the formula of Maguire (1962). Five replicates of 20 seeds were used from each lot.

Seeds belonging to the two lots were packed in tulle-type nylon bags (10 x 13 cm) and allowed to soak in saturated desiccators (95-99% RH) until they reached hydration levels of approximately 10, 15, 20 and 25% water content, at temperatures of 15 and 25 °C. The time necessary for the soaked seeds to reach the desired water content was calculated using the initial water content of the seeds and the weight of each sample, from the following equation described by Cromarty et al. (1990):

$$M = \frac{(100 - CA_1)}{(100 - CA_2)} \times Mi$$

where: M = weight of the desired water content (g), Mi = weight of the original water content (g) = CA_1 original water content (% wet basis), CA_2 = desired water content (% wet basis).

The time required for seeds to reach the proposed hydration levels varied with the lot and temperature (Table 1). After reaching these hydration levels, samples of seed cotyledons from both lots were dried in an air-flow oven at 45 °C for 24 hours and stored in airtight glass tubes, which were kept at -20 °C until the extraction and quantification of the reserves.

Table 1. Time necessary for seeds of lots I and II to reach the hydration levels proposed, at 15 and 25 ºC.

Temperature (ºC)	Lot I		Lot II	
	Hydration (%)	Time (hours)	Hydration (%)	Time (hours)
15	10	70	10	22
	15	262	15	144
	20	588	20	334
	25	956	25	692
25	10	70	10	22
	15	197	15	70
	20	550	20	262
	25	868	25	604

The determination of the lipids was obtained by extracting the oil contained in the seeds of the two lots in a Soxhlet apparatus, and then estimating the oil content from the weight difference between the seed material and the crushed, defatted material, according to the procedures described by Silva (1990). The seed samples were ground up and placed in filter paper cartridges, weighed and transferred to the equipment, being kept at reflux with hexane for 24 hours. They were then dried at 45 °C for 60 minutes and weighed again and the result expressed as a percentage of lipids (oils) extracted.

For the extraction of soluble sugars, degreased samples were maintained in 80% ethanol in a water bath at 75 °C for 30 minutes and centrifuged at 16,000 *xg* for 10 minutes to collect the supernatant liquid. This process was repeated three times (Buckeridge and Dietrich, 1990) and after the extractions, the samples were resuspended in 1.0 mL of distilled water and the mixture was centrifuged, and the supernatant liquid removed and used to quantify the sugar with the colorimetric method (Dubois et al., 1956). The resulting precipitate from the extraction of the soluble sugars was dried at 45 °C for 24 hours and the starch digested with 1.0 mL of 35% perchloric acid for 15 minutes, and then quantified by the colorimetric method (Passos, 1996).

The extraction of soluble proteins was performed using sodium acetate buffer 100 mM, pH 5.0, 25 ºC, as the extraction solution. They were measured according to Bradford (1976), using bovine serum albumin (BSA) as standard.

The experimental design was completely randomized, in a 4x2 factorial within each plot (four hydration levels and two temperatures), plus the control (unhydrated seeds) as an additional treatment. Analyses of variance were performed and the average germination and GSI were compared by the Tukey test at 5% probability. The organic reserves of lipids, soluble carbohydrates, starch and soluble proteins were evaluated using linear and nonlinear regression models, selected in accordance with the highest correlation coefficient and lowest standard error of the mean. The significance of the regressions was tested by the t-test at 5%. The statistical program used was Statistica 8.0 (Statsoft, 2008).

Results and Discussion

Dalbergia nigra seeds had initial water contents of 7.92% for lot I and 8.98% for lot II (Table 2). Germination of the seeds from lots I and II were 80% and 33% respectively. Lot I also had a higher average value for GSI than lot II, with values of 1.85 and 0.74, respectively.

Table 2. Initial moisture percentage (U), germination (G) and germination speed index (GSI) of *Dalbergia nigra* seeds belonging to lots I and II.

	U (%)	G (%)	GSI
Lot I	7.92	80 a	1.85 a
Lot II	8.98	33 b	0.74 b
F Value	-	34.5	36.7
CV (%)	-	21.1	21.0

Means followed by same letter in the column do not differ according to the Tukey test at 5%.

Based on the values for seed germination and GSI for both lots, lot I could be classified as having high vigor and lot II, low vigor. Considering that the seeds of all the trees were harvested at the same time and handled in the same way, the differences in the percentage of germination and GSI between the two lots are probably due to their origin, related to physiological maturity at harvest and environmental conditions of the locality of the mother plants. The quality of seeds from different trees can vary depending on their history, from physiological maturity to storage, including the conditions the seeds are exposed to in the field, before and during harvest, and the drying and processing methods (Popinigis, 1985; Almeida et al., 2007).

Regarding changes in the organic composition of the primary metabolites, it was found that the percentage of lipids did not vary significantly during seed hydration for either of the two lots or temperatures tested (Figure 1). Comparing lots I and II, a slightly higher percentage of lipids was observed in quiescent seeds of lot I, with a mean of 25.2%, while the average for lot II was 23.9%, indicating that lot quality is not related to this type of reserve. Similar results were seen in both lots for lipids between 15 and

25 °C, so that temperature did not affect lipid mobilization at the hydration levels evaluated. Since root protrusion did not occur at these hydration levels, lipid mobilization in the *D. nigra* seeds of lots I and II was not significant and levels were stable.

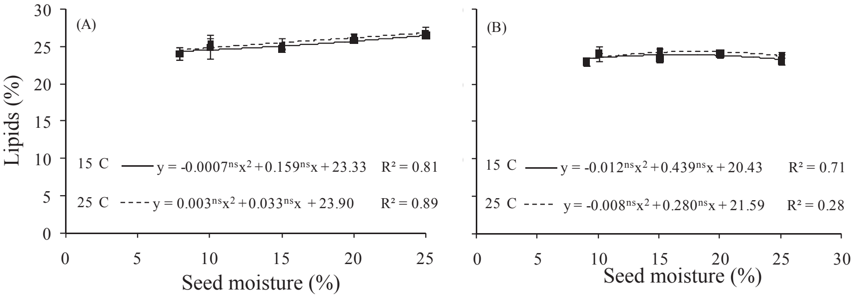

Figure 1. Lipid concentration (%) in lots I (A) and II (B) of *Dalbergia nigra* cotyledons at different levels of water content (10, 15, 20 and 25% water content), hydrated at 15 and 25 °C.
*Value significant at 5%; ns - value not significant at 5%, by t test. The points represent means ± standard deviation.

Lipids are relatively stable energy sources and function as an important reserve in the energy economy of the cell (Campbell and Farrell, 2006). After lipid hydrolysis, the energy necessary for radicle emergence is divided between the storage organs, such as cotyledons and the tube axis reserves, and for early seedling growth and development (Davies and Slack, 1981). In addition, lipase activity, enzymes responsible for the hydrolysis of triglycerides, increases rapidly in the post-germination stage, and is related to the mobilization of lipids (Abigor et al., 2002; Polizelli et al., 2008; Barros et al., 2010.)

The lipids are accumulated in seeds as triglycerides and degraded into glycerol and fatty acids, which are used in the synthesis of sucrose to provide energy and carbon skeletons for seedling growth (Borges and Rena, 1993; Buckeridge et al., 2004). The lipid content of the endosperm of *Jatropha curcas* seeds also remained stable during seed germination, with significant changes only in the post-germination stage, where almost all the oil present was consumed after 96 hours of soaking (Yang et al., 2009.) In *Pangium edule* seeds, the lipid content decreased from 46 to 18.5% during germination, with a faster degradation after hypocotyl emergence (Andarwulan et al., 1999). Similarly, a significant reduction in lipid content was found in seed cotyledons of *Dalbergia misculobium* evaluated at the final stage, when the hypocotyl-radicle measured between 20-50 mm (Silva et al., 1998).

A reduction in the lipid reserves of *Caesalpinia peltophoroides* seeds was observed up to the twentieth day after sowing, indicating the heavy consumption of the seed reserves, suggesting the direct involvement of lipids in the energy supply for germination and seedling establishment (Corte et al., 2006). In the endosperm of *Avena sativa* seeds, lipid mobilization began on the second day after germination, so that on day 10 only 20% of the initial reserves remained in the seeds (Leonova et al., 2010).

The soluble carbohydrates in lots I and II of the *D. nigra* seeds were similar at the two hydration temperatures, with a gradual increase until 15% water content but showing a decreasing trend for 20 and 25% water contents (Figure 2). The initial increase in sugars in the cotyledons may be an accumulation of sucrose, and since these are not readily mobilized, they therefore accumulate, as observed in *Pisum sativum* seeds (Monerri et al., 1986). Increases in sucrose concentrations were observed in the cotyledons of *Vicia faba* seeds until the second day after the start of imbibition, but decreasing significantly after this until day seven (Goyoaga et al., 2011).

A significant reductions in soluble sugars after the 15% water content level in lots I and II suggested that these reserves were used during germination by serving as an energy source, a substrate for respiration and the formation of cell wall protoplasm (Buckeridge et al., 2004). Therefore, seed water absorption stimulates metabolism by activating preexisting enzymes used for digesting hydrolytic reserve substances stored in the seed, which provide nutrients to the embryo and for seedling growth (Borges and Rena, 1993; Sana et al., 2009).

The use of sugars for respiration was observed during the germination of *Schizolobium parahyba*, with a clear reduction during the initial soaking and a subsequent stabilization in the cotyledons between 2 and 8 days (Magalhães et al., 2010). In a study on reserve mobilization in *Aniba rosaeodora* seeds, there was a 30% reduction in soluble sugars when the radicle length was 2-5 cm, with this mobilization being associated with the formation of the first leaf (Lima et al., 2008). Soluble sugar levels also decreased during imbibition of *Senna macranthera* (Borges et al., 2002), *Apuleia leiocarpa* (Pontes et al., 2002) and

Caesalpinia peltophoroides (Corte et al., 2006) seeds, indicating the mobilization of carbohydrates for the germination process.

The reserves stored as starch in *Dalbergia nigra* seeds varied significantly during hydration at 15 to 25 °C for lots I and II (Figure 3). The highest starch values were observed for 15% seed water content, with a tendency to decrease for both lots at higher water content levels. The increase of starch at the 15% water content level is probably due to low use by the embryo. Thus, after the reactivation of metabolism with water levels of 20 and 25%, the starch begins to be removed, even with a low consumption. The weights of the starch contents in the quiescent seeds of *D. nigra* in lots I and II were 114.5 and 121.5 mg.g^{-1} of dry weight, which are low compared to the soluble sugar in lot II (471.2 and 447.6 mg.g^{-1} DM, respectively). Therefore, it is quite likely that this is not the main carbohydrate reserve in this species, as also observed in the leguminous trees *Dalbergia miscolobium*, *C. peltophoroides* and *C. pyramidalis* (Silva et al., 1998, Corte et al., 2006; Dantas et al., 2008a).

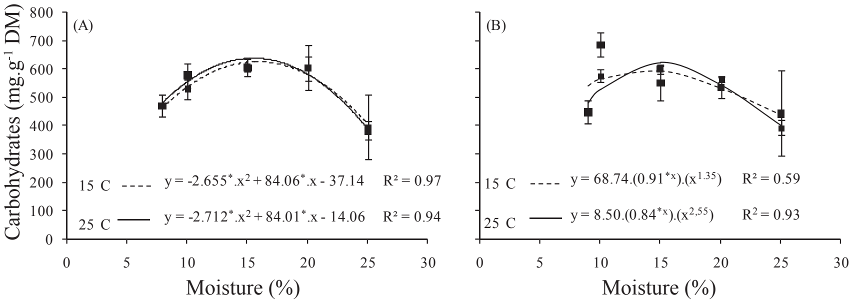

Figure 2. Soluble carbohydrate concentration (mg.g^{-1} of dry weight) in lots I (A) and II (B) of *Dalbergia nigra* cotyledons at different hydration levels (10, 15, 20 and 25% water content), hydrated at 15 and 25 °C.

*Value significant at 5%; ns - value not significant at 5%, by t tSeed moisture; represent means ± standard deviation.

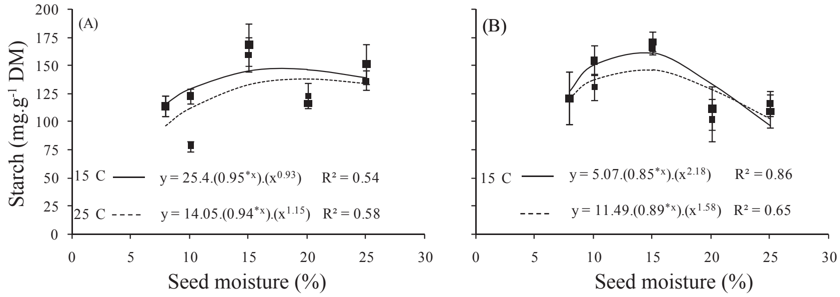

Figure 3. Starch concentration (mg.g^{-1} of dry weight) in lots I (A) and II (B) of *Dalbergia nigra* cotyledons at different moisture levels (10, 15, 20 and 25% water content), hydrated at 15 and 25 °C.

*Value significant at 5%; ns - value not significant at 5%, by t test. The points represent means ± standard deviation.

During germination, the starch granules are broken down into smaller structures, such as maltose, glucose, sucrose and ATP, supplying cells through the action of the enzymes α-amylase, β-amylase and starch phosphorylase (Yamasaki, 2003; Buckeridge et al., 2004). The lower rates of starch mobilization compared to soluble sugars may be due to soluble sugars being the first reserves to be used after imbibition (Ziegler, 1995). Starch hydrolysis provides soluble sugars for respiration during seed germination (Tonini et al., 2010a), supplies energy and helps in the formation of structures in the new seedling. The presence of sugars, such as sucrose and mannitol, inhibits starch mobilization in the cotyledons to the embryo, resulting in a lower production of glucose from starch (Kaur et al., 2005).

Relatively low concentrations of starch reserves were found in *Schinopsis brasiliensis* seeds, with a low consumption after phase II of water uptake during germination (Dantas et al., 2008b). In *Caesalpinia peltophoroides*, starch content showed a slight reduction during germination and seedling growth (Corte et al., 2006). In *Euterpe edulis* seeds, collected from plants growing in the floodplain and on dry land in the Amazon, there was a decrease of around 40% in starch reserves in the seeds from dry land, but there was no difference in the levels of reserves for seeds collected in the floodplain (Gonçalves et al., 2010). The starch content of the cotyledons of *Phaseolus vulgaris* seeds decreased after the start of soaking, and was associated with increased activity by the α-amylase enzyme (Sfaxi-Bousbih et al., 2010).

The soluble protein content of the cotyledons of *Dalbergia nigra* seeds decreased gradually over the whole period of hydration for both lots (Figure 4). Within each lot, similar trends were observed for protein mobilization between 15 and 25 °C. The seeds had a quiescent soluble protein content of 11.04 and 10.85 mg.g^{-1} dry matter for lots I and II, respectively. After hydration up to a level of 25% water content, these initial levels fell to 7.35 mg.g^{-1} for the lot I and 7.70 mg.g^{-1} dry matters for lot II. Henning et al. (2010) observed that more vigorous seeds of *Glycine max* contained higher amounts of soluble protein compared to less vigorous seeds, but with a maximum variation of 4 µg.g^{-1} of dry matter between lots of different physiological qualities.

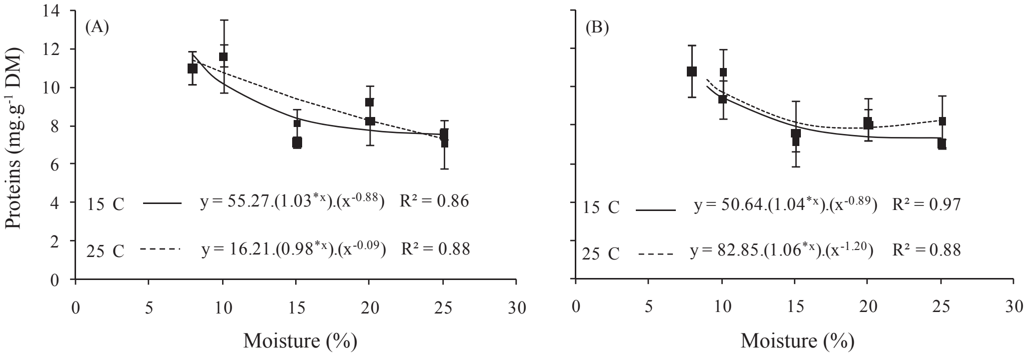

Figure 4. Soluble protein concentration (mg.g^{-1} of dry weight) in lots I (A) and II (B) of *Dalbergia nigra* cotyledons at different water content levels (10, 15, 20 and 25% water content), hydrated at 15 and 25 °C.

*Value significant at 5%; ns - value not significant at 5%, by t test. The points represent means ± standard deviation.

The variations found in protein levels during seed hydration of *D. nigra* indicates that these molecules are hydrolyzed, generating their constituent amino acids, which are used to synthesize new proteins and enzymes and to provide energy from the oxidation of the carbon skeleton after deamination (Buckeridge et al., 2004). Protein mobilization is necessary to meet the demand for amino acids during the early stages of germination and is the first nitrogen mineral nutrient to be used by the seed (Soriano et al., 2011). During the germination process, the development of the embryonic axis and the synthesis of new proteins also depend on the supply of appropriate amino acids derived from the breakdown of storage proteins (Bewley and Black, 1994; Kim et al., 2011).

Proteins appear as the major chemical components responsible for imbibition and an increase in seed size, followed by cellulose and pectin, while starch and lipids interfere less in the process (Copeland and McDonald, 1995). In *Araucaria bidwillii* seeds, quantification of proteins demonstrated a similar distribution of soluble and insoluble proteins in dry seeds, so that during the germination the proteins were degraded from the start of imbibition, with the reduction of soluble proteins being slower than for insoluble proteins (Capocchi et al., 2011).

Protein levels decreased during the first 48 hours of imbibition in *Prosopis juliflora* cotyledons, and were used in the activation of the enzymes needed to break the seed coat during radicle protrusion (Gallão et al., 2007). Whereas soluble protein contents decreased, there was an increase in free amino acid levels in *Vicia faba* cotyledons over a period of seven days after the start of immersion (Kirmizi and Güleryüz, 2006). In different cultivars of *Linumu sitatissimum*, a regular reduction in total protein content was observed when seeds were evaluated under normal and stress conditions (Sebei et al., 2007). The protein content in the testa and endosperm of *Sesbania virgata* seeds tended to decrease the third day after the start of imbibition (Tonini et al., 2010b), as was also observed in seeds of *C. peltophoroides* (Corte et al., 2006).

Conclusions

The time taken to reach the final water content depends on the initial seed water content and not on the temperature or physiological quality of the seed lot.

Changes in soluble carbohydrate, starch, lipid and protein contents in the cotyledons depend on the physiological quality of the seed lot and the seed water content.

The reduction of starch content in cotyledons is pronounced in the less vigorous seed lot.

References

ABIGOR, R.D.; UADIA, P.O.; FOGLIA, T.A.; HAAS, M.J.; SCOTT, K.; SAVARY, B.J. Partial purification and properties of lipase from germinating seeds of *Jatropha curcas* L. *JAOCS*, v.79, n.11, p.1123-1126, 2002. http://www.springerlink.com/index/l2v352l16m043q65.pdf

ALMEIDA, F.A.C.; ALVES, N.M.C.; GOMES, J.P.; SILVA, D.R.S. Determinação do teor de umidade limite de sementes de endro (*Anethum graveolens)* para crioconservação. *Revista de Biologia e Ciências da Terra*, v.7, n.2, p.153-159, 2007. http://redalyc.uaemex.mx/src/inicio/artpdfred.jsp?icve=50007219

ALVARADO, V.; BRADFORD, J.K. A hydrothermal time model explains the cardinal temperatures for seed germination. *Plant, Cell & Environment*, v.25, n.9, p.1061-1071, 2002. http://www.mendeley.com/research/a-hydrothermal-time-model-explains-the-cardinal-temperatures-for-seed-germination

ANDARWULAN, N.; FARDIAZ, D.; WATTIMENA, G.A.; SHETTY, K. Antioxidant activity associated with lipid and phenolic mobilization during seed germination of *Pangium edule* Reinw. *Journal of Agricultural and Food Chemistry*, v.47, p.3158-3163, 1999. http://www.ncbi.nlm.nih.gov/pubmed/10552624

BARROS, M.; FLEURI, L.F.; MACEDO, G.A. Seed lipases: sources, applications and properties – a review. *Brazilian Journal of Chemical Engineering,* v.27, n.1, p.15-29, 2010. http://www.scielo.br/scielo.php?pid=s0104-66322010000100002&script=sci_abstract

BEWLEY, J.D.; BLACK, M. *Seeds: physiology of development and germination*. New York: Plenum Press, 1994. 445 p.

BORGES, E.E.L.; BORGES, R.C.G.; SOARES, C.P.B.; PEREZ, S.C.J.G. Crescimento e mobilização de carboidrato em embrião de sementes de fedegoso (*Senna macranthera* Irwin et Barneby) durante a germinação. *Revista Cerne*, v.8, n.1, p.69-76, 2002. http://redalyc.uaemex.mx/pdf/744/74408107.pdf

BORGES, E.E.L.; RENA, A.B. Germinação de sementes. In: AGUIAR, I.B.; PIÑA-RODRIGUES, F.C.M.; FIGLIOLIA, M.B. (Coord.) *Sementes florestais tropicais*. Brasília: ABRATES, 1993. p.83-136.

BRADFORD, M.M. A rapid and sensitive method for the quantifications of microgram quantities of protein utilizing the principle of protein-dye binding. *Analitycal Biochemistry,* v.72, p.248-254, 1976. http://www.ncbi.nlm.nih.gov/pubmed/942051

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. *Regras para análise de sementes*. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Brasília: MAPA/ACS, 2009. 395p. http://www.agricultura.gov.br/arq_editor/file/laborat%c3%b3rio/sementes/regras%20para%20analise%20de%20sementes.pdf

BUCKERIDGE, M.S.; DIETRICH, S.M.C. Galactomanans from Brazilian legume seeds. *Revista Brasileira de Botânica,* v.13, p.109-112, 1990.

BUCKERIDGE, M.S.; SANTOS, H.P.; TINÉ, M.A.S.; AIDAR, M.P. Mobilização de reservas. In: FERREIRA, AG.; BORGHETTI, F. *Germinação: do básico ao aplicado*. Porto Alegre: Artmed, 2004. 324p.

CAMPBELL, M.K.; FARRELL, S.O. *Bioquímica*. 5. ed. São Paulo: Thomson, 2006. 845p.

CAPOCCHI, A. MUCCILLI, V.; CASANI, S.; FOTI, S.; GALLESCHI, L.; FONTANINI, D. Proteolytic enzymes in storage protein mobilization and cell death of the megagametophyte of *Araucaria bidwillii* Hook. post-germinated seeds. *Planta,* v.233, p.817-830, 2011. http://www.ncbi.nlm.nih.gov/pubmed/21212976

COPELAND, L.O.; McDONALD, M.B. *Principles of seed science and technology.* 3. ed. New York: Chapman e Hall, 1995. 409p.

CORTE, V.B.; BORGES, E.E.L.; PONTES, C.A.; LEITE, I.T.A.; VENTRELLA, M.C.; MATHIAS, A.A. Mobilização de reservas durante a germinação das sementes e crescimento das plântulas de *Caesalpinia peltophoroides* Benth. (Leguminosae Caesalpinoideae). *Revista Árvore*, v.30, n.6, p.941-949, 2006. http://www.scielo.br/scielo.php?pid=s0100-67622006000600009&script=sci_arttext

CROMARTY, A.S.; ELLIS, R.H.; ROBERTS, E.H. *The design of seed storage facilities for genetic conservation*. Rome: International Board of Plant Genetic Resources, 1990. 109p.

DANTAS, B.F.; CORREIA, J.S.; MARINHO, L.B.; ARAGÃO, C.A. Alterações bioquímicas durante a embebição de sementes de catingueira **(***Caesalpinia pyramidalis* Tul.). *Revista Brasileira de Sementes,* v.30, n.1, p.221-227, 2008a. http://www.alice.cnptia.embrapa.br/handle/doc/144048

DANTAS, B.F.; SOARES, F.S.J.; LÚCIO, A.A.; ARAGÃO, C.A. Alterações bioquímicas durante a embebição de sementes de baraúna (*Schinopsis brasiliensis* Engl.). *Revista Brasileira de Sementes*, v.30, n.2, p.214-219, 2008b. http://www.scielo.br/scielo.php?pid=S0101-31222008000200027&script=sci_arttext

DAVIES, H.V.; SLACK, P.T. The control of food mobilization in seeds of dicotyledonous plants**.** *New Phytologist,* v.88, p.41-51, 1981. http://www.jstor.org/pss/2434282

DUBOIS, M.; GILLES, K.A.; HAMILTON, J.K.; REBERS, P.A.; SMITH, F. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, v.28, p.350-356, 1956. http://pubs.acs.org/doi/abs/10.1021/ac60111a017

FERRAZ-GRANDE, F.G.A.; TAKAKI, M. Temperature dependent seed germination of *Dalbergia nigra* Allem (Leguminosae). *Brazilian Archives of Biology and Technology*, v.44, p.401-404, 2001.

GALLÃO, M.I.; VIEIRA, I.G.P.; MENDES, F.N.O.; SOUZA, A.S.N.; BRITO, E.S. Reserve mobilization in mesquite (*Prosopis juliflora*) seed (Leguminosae). *Journal of the Science of Food and Agriculture,* v.87, p.2012-2018, 2007. http://onlinelibrary.wiley.com/doi/10.1002/jsfa.2936/

GONÇALVES, J.F.C.; LIMA, R.B.S.; FERNANDES, A.V.; BORGES, E.E.L.; BUCKERIDGE, M.S. Physiological and biochemical characterization of the assai palm (*Euterpe oleracea* Mart.) during seed germination and seedling growth under aerobic and anaerobic conditions. *Revista Árvore*, v.34, n.6, p.1045-1053, 2010. http://www.scielo.br/scielo.php?pid=s0100-67622010000600010&script=sci_arttext

GOYOAGA, C.; BURBANO, C.; CUADRADO, C.; ROMERO, C.; GUILLAMO, E.; VARELA, A.; PEDROSA, M.M.; MUZQUIA, M. Content and distribution of protein, sugars and inositol phosphates during the germination and seedling growth of two cultivars of *Vicia faba*. *Journal of Food Composition and Analysis,* v.24, p.391-397, 2011. http://www.sciencedirect.com/science/article/pii/s088915751000284x

HENNING, F.A.; MERTZ, L.M.; JACOB JUNIOR, A.; MACHADO, R.D.; FISS, G.; ZIMMER, P.D. Composição química e mobilização de reservas em sementes de soja de alto e baixo vigor. *Bragantia*, v.69, n.3, p.727-734, 2010. http://redalyc.uaemex.mx/src/inicio/artpdfred.jsp?icve=90816059026

IBAMA. *Lista Oficial de Flora ameaçada de extinção*. http://www.ibama.gov.br/flora. Acesso em: 02 mai. 2010.

KAUR, N.; KAUR, H.; GUPTA, A.K. Effect of exogenous sucrose on the enzymes of starch degradation and sucrose metabolism in cowpea (*Vigna unguiculata* L.) seedlings. *Indian Journal of Biochemistry & Biophysics*, v.42, p.295-300, 2005. http://nopr.niscair.res.in/handle/123456789/3537

KERBAUY, G.B. *Fisiologia Vegetal*. Rio de Janeiro: Guanabara Koogan, 2004. 452 p.

KIM, H.T.; CHOI, U.K.; RYU, H.S.; LEE, S.J.; KWON, O.S. Mobilization of storage proteins in soybean seed (*Glycine max* L.) during germination and seedling growth. *Biochimica et Biophysica Acta,* v.1814, p.1178-1187, 2011. http://www.ncbi.nlm.nih.gov/pubmed/21616178

KIRMIZI, S.; GÜLERYÜZ, G. Protein mobilization and proteolytic enzyme activities during seed germination of broad bean (*Vicia faba* L.). *Zeitschrift für Naturforschung C,* v.61, p.222-226, 2006. http://www.ncbi.nlm.nih.gov/pubmed/16729580

LEONOVA, S.; GRIMBERG, A.; MARTTILA, S.; STYMNE, S.; CARLSSON, A.S. Mobilization of lipid reserves during germination of oat (*Avena sativa* L.), a cereal rich in endosperm oil. *Journal of Experimental Botany*, v.61, n.11, p.3089-3099, 2010. http://jxb.oxfordjournals.org/content/61/11/3089

LIMA, R.B.S.; GONÇALVES, J.F.C.; PANDO, S.C.; FERNANDES, A.V.; SANTOS, A.L.W. Primary metabolite mobilization during germination in rosewood (*Aniba rosaeodora* Ducke) seeds. *Revista Árvore*, v.32, n.1, p.19-25, 2008. http://www.scielo.br/scielo.php?script=sciarttext&pid=s0100-67622008000100003

LONG, R.L.; WILKLIAMS, K.; GRIFFITHS, E.M.; FLEMATTI, G.R.; MERRITT, D.J.; STEVENS, J.C.; TURNER, S.R.; POWLES, S.B.; DIXON, K.W. Prior hydration of *Brassica tournefortii* seeds reduces the stimulatory effect of karrikinolide on germination and increases seed sensitivity to abscisic acid. *Annals of Botany*, v.105, p.1063-1070, 2010. http://aob.oxfordjournals.org/content/105/6/1063.short

LORENZI, H. *Árvores brasileiras: manual de identificação e cultivo de plantas arbóreas nativas do Brasil.* Nova Odessa: Plantarum, 1992. 382 p.

MAGALHÃES, S.R.; BORGES, E.E.L.; BERGER, A.P.A. Mobilização de reservas no eixo embrionário e nos cotilédones de sementes de *Schizolobium parahyba* (Vell.) S.F. Blake durante a germinação. *Ciência Florestal*, v.20, n.4, p.589-595, 2010. http://cascavel.ufsm.br/revistas/ojs-2.2.2/index.php/cienciaflorestal/article/view/2417

MAGUIRE, J.D. Speed of germination: aid in selection and evaluating or seedling emergence and vigour. *Crop Science*, v.2, n.2, p.176-177, 1962.

MONERRI, C.; GARCIA-LUIS, A.; GUARDIOLA, J.L. Sugar and starch changes in pea cotyledons during germination. *Physiologia Plantarum*, v.67, p.49-54, 1986. http://onlinelibrary.wiley.com/doi/10.1111/j.1399-3054.1986.tb01261.x/

NONOGAKI, H.; CHEN, F.; BRADFORD, K.J. Mechanisms and genes involved in germination *sensu stricto*. In: BRADFORD, K.J.; NONOGAKI, H. *Seed development, dormancy and germination*. Ames: Blackwell Publishing, 2007. p.264-304.

PASSOS, L. P. *Métodos analíticos e laboratoriais em fisiologia vegetal.* Coronel Pacheco: EMBRAPA-CNPGL, 1996. 223p.

POLIZELLI, P.P.; FACCHINI, F.D.A.; CABRAL, H.; BONILLA-RODRIGUES, G.O. A new lipase isolated from oleaginous seeds from *Pachira aquatica* (Bombacaceae). *Applied Biochemistry and Biotechnology*, v.150, p.233-242, 2008. http://www.springerlink.com/content/828415242p440506/

PONTES, C.A.; BORGES, E.E.L.; BORGES, R.C.G.; SOARES, C.P.B. Mobilização de reservas em sementes de *Apuleia leiocarpa* (Vogel) J.F. Macbr. (garapa) durante a embebição. *Revista Árvore*, v.26, n.5, p.593-601, 2002. http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0100-67622002000500010

POPINIGIS, F. 1985. *Fisiologia de sementes*. 2.ed. Brasília, 289p.

RIZZINI, C.T. *Árvores e madeiras úteis do Brasil: manual de dendrologia brasileira.* São Paulo: Editora da USP, 1972. 294p.

SANA, N.K.; SARKAR, B.C.; AZAD, M.A.K.; HUQUE, M.E.; SHAHA, R.K. Enzyme activities and mobilization of nutrients in brassica (*Brassica* spp.) and wheat (*Triticum aestivum* L.) seeds during germination. *Journal of Biosciences*, v.17, p.101-106, 2009. http://www.banglajol.info/index.php/jbs/article/view/7114

SEBEI, K.; DEBEZ, A.; HERCHI, W.; BOUKHCHINA, S.; KALLEL, H. Germination kinetics and seed reserve mobilization in two flax (*Linumus itatissimum* L.) cultivars under moderate salt stress. *Journal of Plant Biology*, v.50, n.4, p.447-454, 2007. http://www.springerlink.com/content/45x07267p47q6u55/

SFAXI-BOUSBIH, A.; CHAOUI, A.; FERJANI, E.E. Copper affects the cotyledonary carbohydrate status during the germination of bean seed. *Biological Trace Element Research,* v.137, p.110-116, 2010. http://www.springerlink.com/content/g0175057ll144v17/

SILVA, T.R.G.; CORTELAZZO, A.L.; DIETRICH, S.M.C. Variations in storage compounds during germination and early plantlet growth of *Dalbergia miscolobium*. *Revista Brasileira de Fisiologia Vegetal,* v.10, n.2, p.119-124, 1998. http://www.cnpdia.embrapa.br/rbfv/pdfs/download.php?file=v10n2p119.pdf

SILVA, D.J. *Análise de alimentos* - métodos químicos e biológicos. Viçosa, MG: Universidade Federal de Viçosa, 1990. 165p.

SORIANO, D.; OROZCO-SEGOVIA, A.; MARQUEZ-GUZMA, J.; KITAJIMA, K.; BUEN, A.G.; HUANTE, P. Seed reserve composition in 19 tree species of a tropical deciduous forest in Mexico and its relationship to seed germination and seedling growth. *Annals of Botany*, v.107, p.939-951, 2011. http://aob.oxfordjournals.org/content/early/2011/03/07/aob.mcr041

STATSOFT INC. *Statistica data analysis system version 8.0*. Tulsa: Statsoft Inc., 2008.

TONINI, P.P.; PURGATTO, E.; BUCKERIDGE, M.S. Effects of abscisic acid, ethylene and sugars on the mobilization of storage proteins and carbohydrates in seeds of the tropical tree *Sesbania virgata* (Leguminosae). *Annals of Botany*, v.106, p.607-616, 2010a. http://aob.oxfordjournals.org/content/early/2010/08/12/aob.mcq159.full

TONINI, P.P.; CARRARA, T.B.; BUCKERIDGE, M.S. Storage proteins and cell wall mobilization in seeds of *Sesbania virgata* (Cav.) Pers. (Leguminosae). *Trees*, v.24, p.675-684, 2010b. http://www.springerlink.com/content/66835482u535763k/

VARIER, A.; VARI, A.K.; DADLANI, M. The subcellular basis of seed priming. *Current Science*, v.99, n.4, p.450-456, 2010. http://www.mendeley.com/research/subcellular-basis-seed-priming/

WANG, J.; LI, Y.; LO, S.W.; HILLMER, S.; SUN, S.S.M.; ROBINSON, D.G.; JIANG, L. Protein mobilization in germinating mung bean seeds involves vacuolar sorting receptors and multivesicular bodies. *Plant Physiology*, v.143, p.1628-1639, 2007. http://www.plantphysiol.org/content/143/4/1628

YAMASAKI, Y. β-Amylase in germinating millet seeds. *Phytochemistry*, v.64, p.935-939, 2003. http://www.ncbi.nlm.nih.gov/pubmed/14561508

YANG, M.F.; LIU, Y.J.; LIU, Y.; CHEN, H.; CHEN, F.; SHEN, S. Proteomic analysis of oil mobilization in seed germination and postgermination development of *Jatropha curcas*. *Journal of Proteome Research*, v.8, p.1441-1451, 2009. http://pubs.acs.org/doi/abs/10.1021/pr800799s

ZIEGLER, P. Carbohydrate degradation during germination. In: KIGEL, J.; GALILI, G. (Eds.). *Seed development and germination.* New York: Marcel Dekker, 1995. p.447-474.