CHANGES IN SOLUBLE CARBOHYDRATES DURING STORAGE OF *Caesalpinia echinata* LAM. (BRAZILWOOD) SEEDS, AN ENDANGERED LEGUMINOUS TREE FROM THE BRAZILIAN ATLANTIC FOREST

GARCIA, I. S., SOUZA, A., BARBEDO, C. J.,
DIETRICH, S. M. C. and FIGUEIREDO-RIBEIRO, R. C. L.

Instituto de Botânica, C. P. 4005, CEP 01061-970, São Paulo, SP, Brazil

Correspondence to: Rita de Cassia L. Figueiredo Ribeiro, Instituto de Botânica, C. P. 4005, CEP 01061-970, São Paulo, SP, Brazil, e-mail: ritarib@usp.br

Received August 27, 2004 – October 7, 2004 – Distributed May 31, 2006

(With 2 figures)

ABSTRACT

Caesalpinia echinata seeds stored in laboratory environmental conditions lose their viability in one month whilst under low temperatures germination is maintained for 18 months of storage. These seeds are tolerant to desiccation, keeping their viability up to 0.08 $gH_2O.gDW^{-1}$. Since soluble carbohydrates are believed to be involved with desiccation tolerance and seed storability, the aim of this work is to analyze the content and composition of soluble carbohydrates in *C. echinata* seeds during storage in paper bags (PB) and glass flasks (GF) at laboratory room (RT) and cool (CT) temperatures. In freshly harvested seeds, total soluble carbohydrates comprised approximately 10% of the dry weight, decreasing to *ca.* 8% over 18 months of storage at RT. In seeds stored at CT, sugars varied differently decreasing initially and being restored at the end of the analysis period. The main neutral sugars in seeds from all treatments were sucrose, fructose and glucose. Raffinose and stachyose were present as traces. Free *myo*-inositol and other cyclitols were also detected. The main tendency observed was the variation in levels of both glucose and fructose in relation to sucrose, the highest levels of monosaccharides which were found in seeds stored at CT. The values of glucose and fructose were practically constant in seeds stored in paper bags for 18 months at CT, decreasing consistently in the other treatments, mainly at RT. Sucrose contents remained relatively stable. Changes in soluble sugars during storage suggest that the loss of germinability of seeds of *C. echinata* could be associated with low levels of glucose and fructose in relation to sucrose.

Keywords: seed longevity, desiccation tolerance, pernambuco, soluble sugars, cyclitols.

RESUMO

Modificações nos carboidratos solúveis durante o armazenamento de sementes de *Caesalpinia echinata* LAM. (pau-brasil), uma leguminosa arbórea da Mata Atlântica em risco de extinção

Sementes de *Caesalpinia echinata* (pau-brasil) perdem a viabilidade em um mês quando armazenadas no ambiente de laboratório, enquanto a capacidade germinativa é mantida quando armazenadas sob temperturas baixas. O presente trabalho teve como objetivos analisar o conteúdo e a composição dos carboidratos de sementes de *C. echinata* armazenadas em câmara fria (CT) e em temperatura ambiente do laboratório (RT), em duas embalagens distintas (permeável e impermeável), visando a avaliar o envolvimento desses compostos com a capacidade germinativa das sementes. Os resultados mostraram que os carboidratos solúveis são constituídos principalmente de sacarose, glicose, frutose, *myo*-inositol e traços de rafinose e estaquiose, totalizando cerca de 10% da massa seca das sementes. As variações nos carboidratos solúveis foram semelhantes nos dois tipos de embalagem, mas diferentes quanto à temperatura de armazenamento. Em CT, as proporções dos monossacarídeos encontradas nas sementes recém-colhidas foram mantidas por cerca de 18 meses de armazenamento, coincidindo com alta porcentagem de germinação (80%). Nas armazenadas em RT houve redução expressiva nas proporções de glicose e frutose e perda completa da germinabilidade. O conteúdo de sacarose se manteve relativamente estável durante todo o período de análise. Os resultados indicam que a perda da germinabilidade de sementes de *C. echinata* está associada à diminuição dos níveis de glicose e frutose em relação aos níveis de sacarose.

Palavras-chave: longevidade de sementes, tolerância à dessecação, pau-brasil, açúcares solúveis, ciclitóis.

INTRODUCTION

Caesalpinia echinata Lam. (Brazilwood, Pernambuco) is a Brazilian leguminous tree rarely found naturally in the moist coastal forests of Eastern Brazil, its original habitat. The species is at risk of extinction (Ibama, 1992) due to the economic exploitation of its heartwood, used as dyestuff in the past and, today still utilized to manufacture high-quality bows of stringed instruments (Lewis, 1998; Rocha, 2004). A galactose/lactose-binding lectin was isolated from *C. echinata* seeds and was shown to have a pro-inflammatory activity inducing the neutrophil migration independently and dependently of resident cells in rats (Alencar *et al*., 2002). Furthermore, various proteinase inhibitors have also been isolated from these seeds, including tripsin, elastase and human plasma kallikrein inhibitors (Oliveira *et al*., 2002). More recently, Cruz-Silva *et al*. (2004) showed that the elastase inhibitor and one kallikrein inhibitor from seeds of *C. echinata* were active in reducing the edema formation in rabbit lungs and suggested that these seeds could be a useful plant material to study the role of these important enzymes in pathophysiological processes.

Brazilwood seeds lose their viability in approximately one month in a natural environment while stored under low temperatures (6-8 °C) in paper bags, germination is maintained up to 80% after 18 months. These seeds are also tolerant to desiccation, keeping their viability up to 0.08 g $H_2O.gDW^{-1}$ (7-8% wet basis) after drying at 40-50 °C (Barbedo *et al*., 2002).

Soluble carbohydrates are an important component involved in desiccation tolerance during seed maturation and storage (Obendorf, 1997). Raffinose, stachyose and verbascose are galactosyl sucrose oligosaccharides of the raffinose family (RFO) that rank next to sucrose in their distribution in the plant kingdom and are accumulated during seed development in a number of food and feed seed crops (Amuti & Pollard, 1977). They are thought to be associated with the storability of seeds as well as other physiological roles (Horbowicz *et al*., 1998 and refs. therein). Free cyclitols and mainly galactosyl cyclitols are also accumulated in some seeds, as are raffinose and stachyose (Obendorf, 1997; Peterbauer & Richter, 2001).

The galactosyl sucrose and galactosyl cyclitol carbohydrates in maturing seeds have been said to be non-toxic and non-reducing forms of storage products for the seeds, also contributing to the structural stability of organelles, membranes, enzymes and other macromolecules as well as the glassy state. Methyl-ether derivatives of cyclitols may also form liquid crystals (Obendorf, 1997; Peterbauer & Richter, 2001).

Despite their tolerance to desiccation, *Caesalpinia echinata* seeds maintain vigor and germinability for long periods only when stored under low temperatures (Barbedo *et al.,* 2002). In the present work we analyzed the content and composition of soluble carbohydrates from seeds stored for 18 months under four different conditions and found a positive relation between monosaccharide levels and germination capacity of seeds stored under cooler temperatures.

MATERIAL AND METHODS

Plant material

Fruit from *Caesalpinia echinata* Lam. (Leguminosae) was collected in Mogi-Guaçu, SP, Brazil (22° 17' S; 47° 03' WG; 600 m of altitude; climate Cwa - Köppen), directly from the trees during November 1999. The fruit was harvested when ripe (brownish green or wholly brown), without visible damage from insects or microorganisms and were kept under laboratory conditions until dehiscence. Collected seeds were mixed at random and separated into three batches, one of them representing freshly harvested seeds and the others stored as described below.

Seed storage and analysis of seed quality

The seeds were stored in paper bags and in Wheaton type glass flasks, which is a common procedure for seed storage, in cold room (CT) and laboratory room temperatures (RT). Temperature and relative humidity of the cold room and laboratory were 6 ± 1 °C and 85% ± 5%, and 25 ± 10 °C and 80% ± 15%, respectively. After 12 and 18 months, seeds from each treatment were sampled and analyzed in four replicates.

The water content was determined by weight loss after drying for 24 h at 105 °C (Brasil, 1992 – Brazilian Rules for Seed Testing) using four replicates of 10 seeds. The results were expressed as g H_2O.g dry mass^{-1} (g.g^{-1}). Germination (root protrusion) tests were carried out at 25 ± 1 °C under 8 h of light, in germination boxes (11 x 11 x 3.5 cm) on two layers of filter paper saturated with water using four replicates of 12 seeds. Germination was evaluated after 10 and 20 days.

Extraction and analysis of soluble carbohydrates

For each treatment, three replicates of 10 seeds each were sampled for sugar extraction and analysis. Soluble carbohydrates were extracted from samples of whole seeds homogenized with a mortar and pestle in 10 mL of 80% ethanol (v/v) after boiling for 5 min to inactivate the enzymes. After centrifugation for 5 min at 1000 *g*, the residue was re-extracted twice with 80% ethanol for 5 min at 80 °C and one time with water for 5 min at 60 °C. The ethanolic and aqueous extracts were pooled and concentrated at 35 °C in a rotary evaporator to a small volume. This was considered as the crude extract.

Total carbohydrates in the extracts were estimated colorimetrically by the phenol-sulphuric method (Dubois *et al*., 1956) using glucose as a standard and were expressed as mg per g of dry weight (mg.gDW^{-1}).

After deionization using a column consisting of equal amounts of Dowex–1 (Cl^- form) and Dowex–50W (H^+ form), neutral soluble carbohydrates were analyzed by high performance anion exchange chromatography coupled with pulsed amperometric detection (HPAEC/PAD) using a 4 x 250 mm CarboPac PA-1 column on a Dionex System Mod. DX 300 (USA), with a linear gradient of 25 mol.m^{-3} to 500 mol.m^{-3} sodium acetate in 150 mol.m^{-3} sodium hydroxide at a flow rate of 1 cm^3.min^{-1}, according to Itaya *et al*. (1997). Sugars were identified by co-chromatography with authentic standards and quantified through the calibration program of the Dionex Computer Interface (USA) to determine the range of the linear response of each sugar.

RESULTS AND DISCUSSION

The mature seeds of *Caesalpinia echinata* are composed of 40-50% non-structural carbohydrates, 8-10% proteins and 20% fats. The majority of the carbohydrate portion is attributed to starch, constituting 30-40% of the seed's dry weight (unpublished). Soluble carbohydrates of the embryos (axis plus cotyledons) represented *ca*. 10% of the dry weight basis and are composed mainly of sucrose, glucose and fructose. Cyclitols, raffinose and stachyose were also present, but in lower proportions (Fig. 1).

The water content of freshly harvested *Caesalpinia echinata* seeds was 0.15 g.g^{-1} and the seed viability based on the germination capacity was 100% (Table 1). The behavior of these seeds during storage was reported previously and revealed that storage at cool temperatures (CT) for 18 months largely prevented the loss of viability shown by the seeds when stored at room temperatures (RT) (Barbedo *et al*., 2002). This observation was confirmed in the present study (Table 1).

In general, higher levels of free sugars were maintained when the seeds were stored under lower temperatures. As shown in Fig. 2 the total content of soluble carbohydrates in freshly harvested seeds comprised about 10% on a dry weight basis, decreasing to *ca.* 8% during 18 months of storage at RT. The content of soluble sugars from seeds stored at CT varied differently, decreasing initially and being restored by the end of the analysis period. These variations in the content of sugars could be associated with increased solubilization of reserves, as reported for other seeds with orthodox behavior at physiological maturity (Nkang, 2002). The results could also be related to the increased stability of enzymes such as invertase or amylases that are known to remain active under cool temperatures.

Changes in the levels of individual free sugars were determined after HPAEC/PAD analysis. Table 1 shows that sucrose was the main soluble

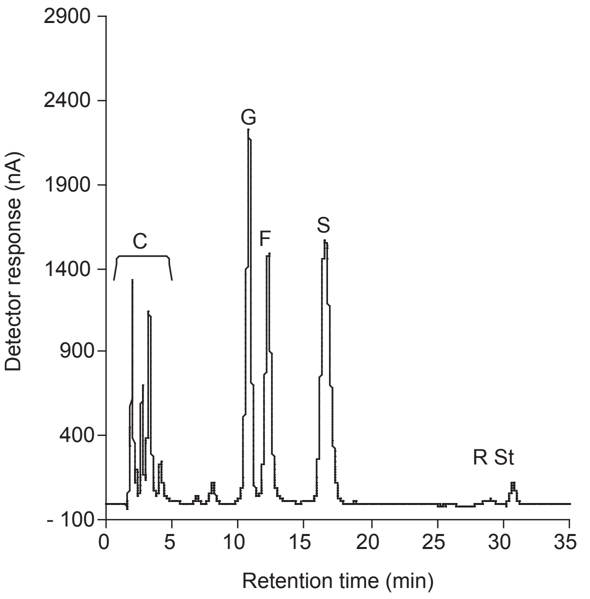

Fig. 1 — HPAEC/PAD profile of soluble carbohydrates from freshly harvested seeds of C*aesalpinia echinata*. C - cyclitols; G - glucose; F - fructose; S - sucrose; R - raffinose; St - stachyose.

TABLE 1
Germination (% in day 20), water content ($gH_2O.gDW^{-1}$) and concentration of individual sugars ($mg.gDW^{-1}$) in *C. echinata* seeds during storage. CT = cold room; RT = room temperature; PB = paper bag; GF = glass flask; G = germination; FRU = fructose; GLU = glucose; SUC = sucrose; RAF = raffinose; ST = stachyose; MYO = *myo*-inositol; WC = water content.

Storage	Time of storage	G	Concentration of sugars ($mg.gDW^{-1}$)						WC
condition	**(months)**	**(%)**	**FRU**	**GLU**	**SUC**	**RAF**	**ST**	**MYO**	**($gH_20.gDW^{-1}$)**
Initial (T0)	0	100	10.6 ± 0.6	10.8 ± 0.7	26.8 ± 1.4	-	0.78 ± 0.21	1.74 ± 0.14	0.15
CT, PB	12	94	10.1 ± 0.8	10.4 ± 0.7	21.4 ± 0.7	-	0.40 ± 0.05	2.52 ± 0.26	*
	18	81	11.3 ± 0.5	10.8 ± 0.3	27.0 ± 0.4	-	0.30 ± 0.09	4.26 ± 0.06	0.10
CT, GF	12	60	9.1 ± 0.1	8.8 ± 0.2	27.5 ± 1.2	-	0.20 ± 0.02	2.60 ± 0.26	0.12
	18	6	7.3 ± 0.2	7.2 ± 0.2	33.5 ± 1.8	-	0.20 ± 0.04	2.36 ± 0.08	0.11
RT, PB	12	0	4.1 ± 0.0	4.2 ± 0.0	28.5 ± 0.1	1.3 ± 0.1	-	2.06 ± 0.02	0.12
	18	0	3.0 ± 0.0	3.0 ± 0.0	34.5 ± 0.2	1.8 ± 0.4	-	5.09 ± 0.02	0.12
RT, GF	12	0	2.6 ± 0.0	2.9 ± 0.0	28.0 ± 0.2	2.0 ± 0.2	-	3.42 ± 0.12	0.15
	18	0	3.4 ± 0.2	3.5 ± 0.2	33.7 ± 1.6	2.7 ± 0.3	-	6.97 ± 0.32	0.15

Values are average of triplicate determinations ± SD; (-) = not detected; (*) = not measured.

carbohydrate from *C. echinata* seeds and its content varied from *ca*. 21 $mg.gDW^{-1}$ to 34 $mg.gDW^{-1}$. The highest values obtained for glucose and fructose ranged from *ca*. 3.0 $mg.gDW^{-1}$ to 11 $mg.gDW^{-1}$ and were equimolar suggesting that their variations are related to the metabolism of sucrose.

The values obtained for both glucose and fructose were maintained practically constant in seeds stored for 18 months at CT mainly in paper bags, decreasing in seeds stored at RT. Changes in soluble sugars during storage at CT and RT indicate that the loss of germinability is accompanied by a reduction in the levels of glucose and fructose leading to an increase in the hexose to sucrose ratio. The decline in the vigor of *Zea mays* embryo was also associated with a marked decline in monosaccharides and in raffinose. In these seeds sucrose content remained relatively stable (Bernal-Lugo & Leopold, 1992), similarly to what was found in the *C. echinata* seeds. In *Erythrina caffra* seeds, the high contents of inositols and monosaccharides were associated to the protection of plasma membranes from the effects of low temperatures (Nkang, 2002). In *Vicia faba*, higher levels of hexoses induced the formation of transfer cells that are involved in the nutrition of the legume embryos, whereas high sucrose concentrations are inhibitory (Borisjuk *et al*., 2003). Thus, it can be suggested that in *C. echinata* seeds stored under conditions (RT) leading to low hexose/sucrose ratio, metabolic and/or structural changes could occur, preventing growth after inhibition.

The oligosaccharide: sucrose (O:S) ratio calculated for seeds stored for different periods and conditions (Table 2) ranged from 0.01 to 0.08, corresponding to values calculated for seeds sensitive to desiccation (Lin & Huang, 1994). The contents of total soluble sugars and of sucrose and the ratio O:S in *C. echinata* seeds were not correlated with the tolerance to desiccation observed for

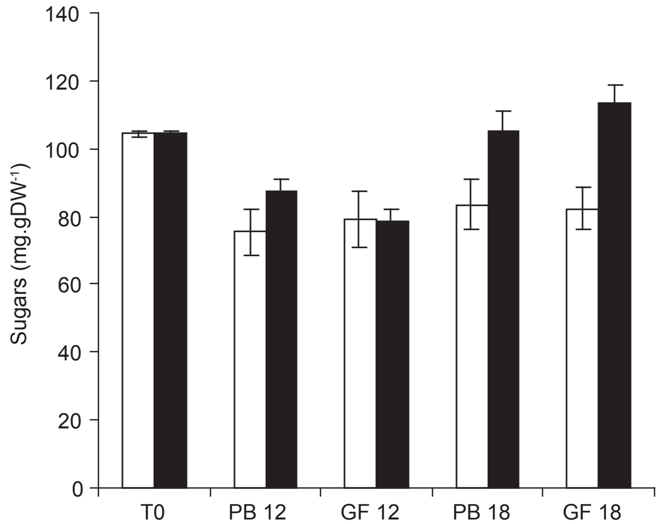

Fig. 2 — Total contents (mg.gDW^{-1}) of soluble carbohydrates from freshly harvested seeds (T0) of *Caesalpinia echinata* and seeds stored for 12 and 18 months at room temperature (☐) and in cold room (■) in paper bags (PB) and in glass flasks (GF). Bars indicate the standard error.

TABLE 2
Soluble sugar proportion in seeds of *C. echinata* during storage. O:T = Oligosaccharide: total sugars; O:S = oligosaccharide: sucrose; G + F: S = glucose + frutose: sucrose. CT = cold room; RT = room temperature; PB = paper bag; GF = glass flask; G = germination on the 20th day.

Storage condition	Time of storage (months)	G (%)	Sugar ratio		
			O:T	O:S	G + F: S
Initial (T0)	0	100	0.02	0.03	0.80
CT, PB	12	94	0.01	0.02	0.96
	18	81	0.01	0.01	0.82
CT, GF	12	60	< 0.01	0.01	0.65
	18	6	< 0.01	0.01	0.43
RT, PB	12	0	0.03	0.05	0.29
	18	0	0.04	0.05	0.17
RT, GF	12	0	0.06	0.07	0.20
	18	0	0.06	0.08	0.20

this species (Barbedo *et al*., 2002). According to Obendorf (1997) in orthodox seeds, the lower the mass ratio of sucrose to soluble oligosaccharides, the lower the storability. Results obtained for *C. echinata* suggest that the proportions of sucrose and galactosyl sucrose oligosaccharides alone do not explain its tolerance to seed desiccation.

In *Fagopyrum esculentum* (buckwheat), instead of α-galactosyl sucrose oligosaccharides, the seeds accumulate α-galactosyl D-*chiro*-inositols which may play a role in desiccation tolerance and storability (Horbowicz *et al*., 1998; Steadman *et al*., 2000). Interesting variations in buckwheat embryos of seeds grown in cooler temperatures were reported by Horbowicz *et al*. (1998) showing that the sucrose was decreased and stachyose and the galactosyl cyclitol fagopyritol B1 increased. A preliminary analysis of maturing *C. echinata* seeds by high resolution gas chromatography coupled with mass spectrometry (kindly performed by A. Richter, University of Vienna) revealed the presence of substantial amounts of various cyclitols as pinitol, ciceritol, ononitol and galactopinitol A and B, which could be an indication of the presence of a galactosyl cyclitol series with similar functions of the RFO. Galactosyl cyclitols are often found in similar or even higher amounts than RFO in seeds of many important grain legumes, such as lentils, chickpeas and soybeans (Peterbauer & Richter, 1998 and ref. therein) and share some common functions, including participating in the acquisition of desiccation tolerance (Peterbauer & Richter, 2001 and ref. therein).

The differences found in soluble sugars and in the germination capacity of *C. echinata* seeds stored in paper bags and in glass flasks also suggest that the longevity of the seeds could be related to their respiration rate, expected to be different under the two storage conditions. Recently, Teixeira *et al.* (2004) reported the presence of paracytic stomata in the seed coat of *C. echinata.* This feature could account for an increase in the seed coat permeability, subjecting the embryo to fluctuations in O_2 concentrations, which are known to affect the activity of invertase and sucrose synthase (Rolletschek *et al*., 2003). Interconversion of sugars may also occur during storage as the alpha-galactosidase and invertase activities were detected in dry seeds of cotton (Shiroya 1963 in Bernal-Lugo & Leopold, 1992).

Analyses of carbohydrate related enzymes and of seed structure during storage at RT and CT in dry *C. echinata* seeds are under investigation. These studies will contribute to the development of strategies for the conservation of this endangered tree species and will also provide new information concerning the biology of dry seeds in one of the most important plant families of the tropical Atlantic Forest.

Acknowledgments — Thanks are due to Dr Andreas Anatol Richter from the University of Vienna for helpful discussions and the critical review of the manuscript. I. S. Garcia thanks CNPq for the fellowship within the PIBIC Program. C. J. Barbedo, R. C. L. Figueiredo-Ribeiro and S. M. C. Dietrich are researchers associated with CNPq. This work was supported by the State of São Paulo Research Foundation (FAPESP), Grant 2000/06422-4.

REFERENCES

ALENCAR, V. B. M., MELO, S. C., ALBUQUERQUE, K. F., FIGUEIREDO, I. S. T., TUPINAMBÁ, D. D., FREITAS, B. T., ALENCAR, N. M. N., SAMPAIO, A. H., RIBEIRO, R. A. & CAVADA, B. S., 2002, Pro-inflammatory effect of galactose binding lectin isolated from *Caesalpinia echinata* Lam. seeds. *In*: Thorkild C. Boo-Hansen, (Ed.), *Lectins, Biology, Biochemistry, Clinical Biochemistry* 16. Lemchesvej (DK): TEXTOP Publisher.

AMUTI, K. S. & POLLARD, C. J., 1977, Soluble carbohydrates of dry and developing seeds. *Phytochemistry 16*: 529-532.

BARBEDO, C. J., BILIA, D. A. & FIGUEIREDO-RIBEIRO, R. C. L., 2002, Tolerância à dessecação e armazenamento de sementes de *Caesalpinia echinata* Lam. (pau-brasil), espécie da Mata Atlântica. *Revta Brasil Bot., 25*: 431-439.

BERNAL-LUGO, I. & LEOPOLD, A. C., 1992, Changes in soluble carbohydrates during seed storage. *Plant Physiol., 98*: 1207-1210.

BORISJUK, L., WANG, T. L., ROLLETSCHEK, H., WOBUS, U. & WEBER, H., 2003, The failure of the embryonic epidermis affects seed maturation and embryo growth in pea, pp. 33-40. *In*: G. Nicolás, K. J. Bradford, D. Côme, and H. W. Pritchard (eds.), *The Biology of Seeds, Recent Research Advances*. London, UK, Cab International Publishing, 472p.

BRASIL. MINISTÉRIO DA AGRICULTURA, 1992, *Regras para análise de sementes*. Brasília, BR: SNDA/DNDV/CLAV.

CRUZ-SILVA, I., GOZZO, A. J., NUNES, V. A., NEUHOF, C., NEUHOF, H. & ARAÚJO, M. S., 2004, Effect of inhibitors from *Caesalpinia echinata* on pulmonary edema. *Proc. Braz. Soc. Biochem. Mol. Biol., 33*: 1.

DUBOIS, N., GILLES, K. A., HAMILTON, J. K., REBERS, P. A. & SMITH, F., 1956, Colorimetric method for determination of sugars and related substances. *Anal. Chem., 28*: 350-356.

HORBOWICZ, M., BRENAC, P. & OBENDORF, R. L., 1998, Fagopyritol B1, O-α-D-galactopyranosyl-(1→2)-D-*chiro*-

inositol, a galactosyl cyclitol in maturing buckwheat seeds associated with desiccation tolerance. *Planta, 205*: 1-11.

IBAMA, 1992, Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (Brasil). Portaria 37N, de 3 de abril de 1992 (www.ibama.gov.br).

ITAYA, N. M., BUCKERIDGE, M. S. & FIGUEIREDO-RIBEIRO, R. C. L., 1997, Biosynthesis *in vitro* of high molecular mass fructan by cell-free extracts from tuberous roots of *Viguiera discolor* (Asteraceae). *New Phytol., 136*: 53-60.

LEWIS, G. P., 1998, *Caesalpinia. A Revision of the Poincianella-Erythrostemon group*. Kew, UK: Royal Botanic Gardens.

LIN, T. P. & HUANG, N. H., 1994, The relationship between carbohydrate composition of some tree seeds and their longevity. *J. Exp. Bot., 45*: 1289-1294.

NKANG, A., 2002, Carbohydrate composition during seed development and germination in two sub-tropical tree species (*Erythrina caffra* and *Guilfoylia monostylis*). *J. Plant. Physiol., 159*: 473-483.

OBENDORF, R. L., 1997, Oligosaccharides and galactosyl cyclitols in seed desiccation tolerance. *Seed Sci. Res., 7*: 63-74.

OLIVEIRA, L. G., GOZZO, A. J., NUNES, V. A., CRUZ-SILVA, I., SAMPAIO, M. U., SAMPAIO, C. A. M. & ARAÚJO, M. S., 2002, Inibidores de proteases encontrados em sementes de *Caesalpinia echinata* (pau-brasil) – isolamento e caracterização do inibidor de tripsina. *Revta Brasil. Farmac, 12*: 72-74.

PETERBAUER, T. & RICHTER, A., 1998, Galactosyl ononitol and stachyose synthesis in seeds of adzuki bean. Purification and characterization of stachyose synthase. *Plant Physiol., 117*: 165-172.

PETERBAUER, T. & RICHTER, A., 2001, Biochemistry and physiology of raffinose family oligosaccharides and galactosyl cyclitols in seeds. *Seed Sci. Res., 11*: 185-197.

ROCHA, Y. T., 2004, *Ibirapitanga: história, distribuição geográfica e conservação do pau-brasil (*Caesalpinia echinata *Lam., Leguminosae) do descobrimento à atualidade*. PhD Thesis, University of São Paulo, São Paulo, Brasil.

ROLLETSCHEK, H., BORISJUK, L., WOBUS, U. & WEBER, H., 2003, Oxygen as a control factor in embryogenesis of legume seeds, pp. 93-99. *In*: G. Nicolás, K. J. Bradford, D. Côme and H. W. Pritchard (eds.), *The Biology of Seeds, Recent Research Advances*. London, UK, Cab International Publishing, 472p.

STEADMAN, K. J., BURGOON, M. S., SCHUSTER, R. L., LEWIS, B. A., EDWARDSON, S. E. & OBENDORF, R. L., 2000, Fagopyritols, D-*chiro*-inositol and other soluble carbohydrates in buckwheat seed milling fractions. *J. Agric. Food Chem., 48*: 2843-2847.

TEIXEIRA, S. P., CARMELLO-GUERREIRO, S. M. & MACHADO, S. R., 2004, Fruit and seed ontogeny related to the seed behavior of two tropical *Caesalpinia* species (Leguminosae). *Bot. J. Linn. Soc.*, 146: 57-70.