Floresta e Ambiente 2023; 30(3): e20220078
https://doi.org/10.1590/2179-8087-FLORAM-2022-0078
ISSN 2179-8087 (online)

ORIGINAL ARTICLE – Silviculture

Mobilization of Storage Reserves in *Dalbergia spruceana* Benth. (Fabaceae) Seeds During Germination at Different Temperatures

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Abstract

Temperature may affect the mobilization and hydrolysis of storage reserves for energy production during seed germination. This study investigated germination performance and reserve mobilization in *Dalbergia spruceana* Benth. seeds incubation at 20, 25, 30, 35, and 40°C. The germination process was favored by incubation at 25 to 35°C and negatively affected at 20 and 40°C. At 35°C there reduction in germination speed, however, without significantly compromising the final germination percentage. The results showed that lipids and proteins are the predominant metabolites in *D. spruceana* seeds. Mobilization of soluble sugars was highest at 25 and 30°C. At 20 and 40°C, mobilization occurred more slowly, negatively affecting germination. This finding, combined with changes in lipid and protein reserves, suggests that lipid and protein hydrolysis products were used for starch synthesis. Reserve mobilization patterns in D. spruceana embryos were influenced by germination temperature, with the highest utilization efficiency occurring between 25 and 35°C.

Keywords: Amazon, Amazon rosewood, biomolecules, cell metabolism, heat stress.

1. INTRODUCTION AND OBJECTIVES

Seed germination can be defined, from a metabolic point of view, as mobilization of storage reserves by hydrolytic enzymes and use of hydrolysis products for the formation of new cellular structures (Alencar et al., 2012; Ataíde et al., 2017). Carbohydrates, proteins, and lipids are the major storage reserves mobilized during germination. Their concentrations in seeds may vary greatly according to forest species (Bewley et al., 2013; Felix et al., 2020a).

Imbibition is one of the first stages of germination. It is necessary for resumption of cell metabolism after a period of metabolic quiescence. Water imbibition leads to an increase in turgor pressure, favoring root expansion (Walters et al., 2017; Oliveira et al., 2020). Some studies have shown that most of the energy used for metabolic processes is consumed during post-germination events and seedling development (Oliveira et al., 2020). Other studies, however, reported that seed reserves may serve as precursors in metabolic pathways necessary for germination, as their levels are altered during embryo development (Tesfay et al., 2016; Ataíde et al., 2017; Santos et al., 2019; Felix et al., 2020a; Reis et al., 2020).

Species differ in the pattern of metabolic events initiated during germination. Mobilization may occur during germination and/or during initial seedling growth. In most cases, carbohydrates and proteins are the first compounds utilized (Yang et al., 2018). Carbohydrates are converted into sucrose for respiration and proteins into amino acids for production of new enzymes and building blocks (Paula et al., 2016; Mazzottini-dos-Santos et al., 2017). As high-energy metabolites, lipids are generally mobilized after proteins and carbohydrates, given the complexity of lipid degradation (Yang et al., 2018).

Seed germination is rigidly controlled by several factors. Temperature, for instance, directly influences metabolic levels during germination and, particularly, the dynamics of reserve

mobilization. When imbibition occurs under optimal temperature conditions, assimilation and translocation of reserves are more efficient (Ataíde et al., 2017). Temperature stress conditions, in contrast, may reduce the activity of enzymes involved in reserve degradation and affect membrane selectivity, precluding the use of essential metabolites for germination (Santos et al., 2019). Temperature can also have an effect on the metabolites used in the germination process as an adaptive strategy to survive thermal variations (Félix et al., 2020; Santos et al., 2020).

Research on native forest species should be intensified and used as a scientific tool for genetic conservation and species propagation. *Dalbergia spruceana* Benth. (Fabaceae), commonly known as Amazon rosewood, jacarandá do Pará, facheiro, and timbó pau, is native to the Amazon Forest, occurring in Amazonas, Amapá, Acre, Rondônia, and Pará States, Brazil (Souza, 2012). It is a small to medium-sized tree up to 10 m tall, the species presents wood with economic potential in function of its hardness, weight and strength, with attractive finishing for the manufacture of furniture and decorative objects (Gonçalves et al., 2012; Souza, 2012). It is component of the secondary vegetation of semidecidual forests, also found in campinarana areas, terra firme forest and Amazon savannah (Carvalho, 1997). Ecologically important in soil recovery, the species shows high potential for forest restoration and rehabilitation programs (Gama e Pinheiro, 2010; Souza, 2012).

Given that germination success is directly associated with species survival, information on metabolic regulation during *D. spruceana* seed germination is important to understanding the use efficiency of essential metabolites, thereby contributing to propagation, conservation, and decision-making in the face of climate changes. We hypothesized that *D. spruceana* seeds are metabolically efficient with regard to mobilization and utilization of organic reserves under specific temperature conditions. This study investigated the dynamics of mobilization and use of organic reserves in *D. spruceana* seeds during germination under different temperature conditions.

2. MATERIAL AND METHODS

D. spruceana fruits were collected from trees in Canaã dos Carajás (06°23′04.55″S 49°50′59.6″W), Pará State, Brazil. According to Koppen's classification, the climate of the region is Aw type, with an average annual precipitation of 2033 mm, average minimum temperatures of 15.6 to 18.3°C, and average maximum temperatures of 34.3 to 38.1°C.

Fruits were processed for seed extraction, and immature, deteriorated, or damaged seeds were discarded. Selected seeds were homogenized, dried to about 8% moisture and stored in a plastic container at 5 °C and 60% relative humidity for 30 days until analysis.

2.1. Germination test

Prior to the germination test, seeds were disinfected with 1% fungicide solution (Captan®) for 60s. Then, seeds were placed to germinate between two sheets of germination paper (Germitest®) in a Petri dish and moistened with deionized water at a water/dry paper ratio of 2.5:1 (w/w) (Brasil, 2009). Plates were incubated in a biochemical oxygen demand incubator at 20, 25, 30, 35, or 40°C for 10 days under constant and alternating light and dark photoperiods (12h). Seeds were considered germinated when the emerged radicle was 2 mm long. Germinated seeds were counted daily, and the results are expressed as germination percentage and germination speed index (GSI) (Maguire, 1962). The equation by Laboriau (1983) was used for the determination of the mean germination time.

2.2. Analysis of reserve mobilization

Seeds were incubated for 4 days under the same conditions as those for the germination test. Embryos were collected every 24 h and analyzed for total lipid, total protein, soluble sugar, and starch contents. After collection, embryos were oven-dried at 45°C for 24 h, placed in hermetically sealed containers, and stored at -20°C until extraction and quantification.

For lipid determination, embryos were ground, and five aliquots of 0.5 g each were placed in filter paper cartridges, weighed, transferred to a Soxhlet apparatus, and extracted with hexane under reflux for 24 h. After extraction, samples were oven-dried at 45 °C for 24 h and weighed. Results are expressed as percentage of lipids extracted in relation to initial sample dry weight (Silva, 1990).

Soluble sugars were extracted in a water bath at 75°C for 30 min. (Buckeridge and Dietrich, 1990). The supernatant was separated by centrifugation at 10,000 × *g* for 5 min, and the extraction process was repeated four times. Starch contents were determined in the precipitate, which was oven-dried at 45°C for 24 h and resuspended in 1.0 mL of distilled water. Five aliquots of 30 mg were subjected to digestion with 1.0 mL of 35% perchloric acid for 15 min. Then, samples were centrifuged at 10,000 × *g* for 5 min, and the supernatant was collected for starch analysis (Passos, 1996). Soluble sugars (5 µL samples) and starch (20 µL samples) were determined by the phenol–sulfuric acid method (Dubois et al., 1956) using glucose (Sigma–Aldrich) as standard.

Total proteins were determined in defatted samples by the micro-Kjeldahl method (Cunniff, 1995), with modifications. Five aliquots of 200 mg were placed in a test tube and mixed with 1.0 g of digestive mixture and 5 mL of 98% sulfuric acid. After digestion in a block digester, the reaction was quenched by the addition of 10 mL of distilled water. Samples were alkalinized with sodium hydroxide (1:1 v/v) and distilled.

The distillate was collected in an Erlenmeyer flask containing 10 mL of 5% boric acid and subjected to titration with 0.05 N hydrochloric acid solution. Total proteins were estimated using a conversion factor of 6.25.

2.3. Statistical analysis

Experiments followed a completely randomized design, with five replications of 20 seeds per treatment. Tukey's test ($p < 0.05$) was used for post-hoc comparisons. Data were subjected to analysis of variance and polynomial regression.

3. RESULTS

The germination process was favored by temperature between 25 and 35°C, with maximum utilization at 30°C, in which 98% germination occurred. The tested temperatures affected the mean germination time of the seeds, with shorter germination times at 25 and 30°C, conditions that also favoreced high germination speed index. At 20 and 40°C, there were significant reductions in germination rates (Figure 1).

In dry *D. spruceana* seeds, lipids were the most abundant reserve compounds, accounting for 39.3% of the embryo dry weight, followed by proteins (38.5%) and carbohydrates (3.1%: 2.5% starch and 0.6% soluble sugars). Regarding the mobilization of these reserves, was significantly influenced by temperature.

The high metabolic activity at 25 and 30°C, stimulated the use of soluble sugars, favoring germination, in which there is clearly intense mobilization of this reserve. Although in the first 24 h of imbibition, soluble sugar concentrations increased at 20 and 35°C, by about 65 e 60%, respectively; then embryos showed a decrease in soluble sugars. At 40 °C, mobilization of soluble sugar occurred from 24 to 48 h of imbibition, followed by stabilization (Figure 2).

Starch concentration increased significantly at all temperatures (Figure 3). At 20ºC, after 48 hours was noticed a significant increase in concentration, followed by stabilization. However, between 25 and 40ºC, concentrantion increased ocurred after 24 hours. At 25, 35 and 40°C, the highest concentration occurred after 72 hours, with an increase of 44, 60 and 72%, respectively. At 30°C, the greatest increase occurred after 96 hours (76%).

Similar patterns of lipid mobilization were observed at different temperatures, with a gradual reduction after 72 h of imbibition, except at 40°C, at which a more significant reduction was observed after 24 h (Figure 4). The initial lipid concentration was 390 mg g^{-1} dry weight, decreasing by 27% after 96 h of imbibition at 40°C.

A significant reduction in protein concentration throughout imbibition was observed at 20, 35, and 40°C. At 25 and 30°C, however, protein concentrations increased after 48 and 72 h of imbibition, respectively (Figure 5).

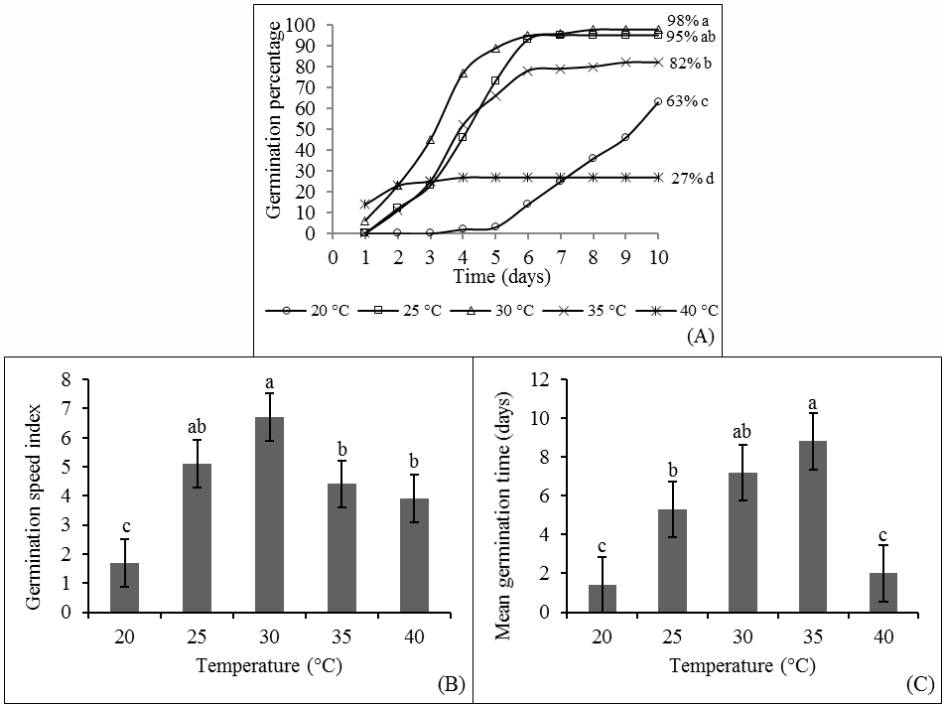

Figure 1. (A) Germination percentage, (B) germination speed index and (C) mean germination time of *Dalbergia spruceana* Benth. seeds incubated for 10 days at different temperatures. Means followed by different letters are significantly different at $p < 0.05$ by Tukey's test. Vertical bars represent the standard error of the mean (n = 5).

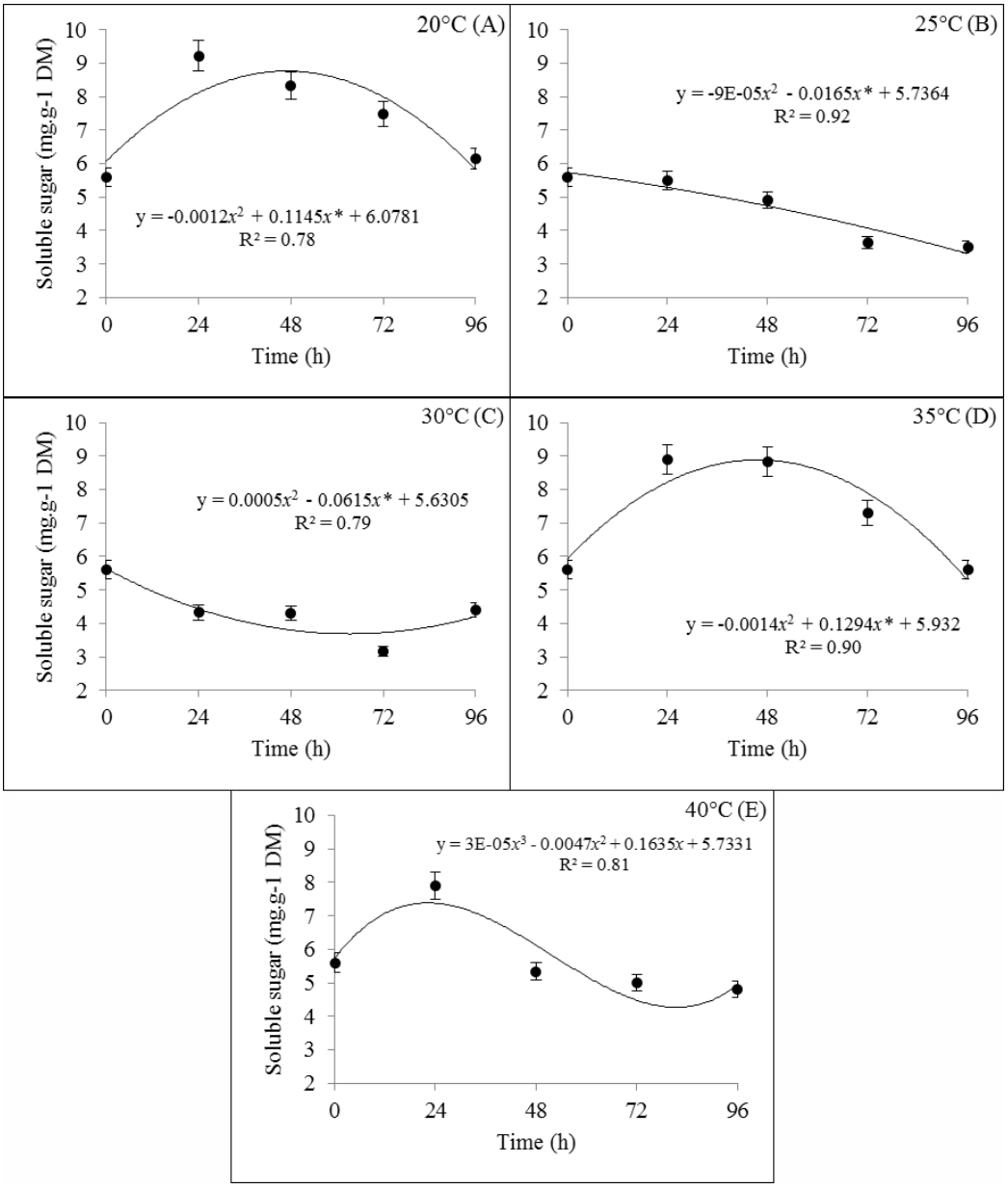

Figure 2. Mobilization of soluble sugars in *Dalbergia spruceana* Benth. embryos during germination at different temperatures. DM, dry matter. An asterisk (*) indicates a significant difference at $p < 0.05$ by Tukey's test. Vertical bars represent the standard error of the mean (n = 5).

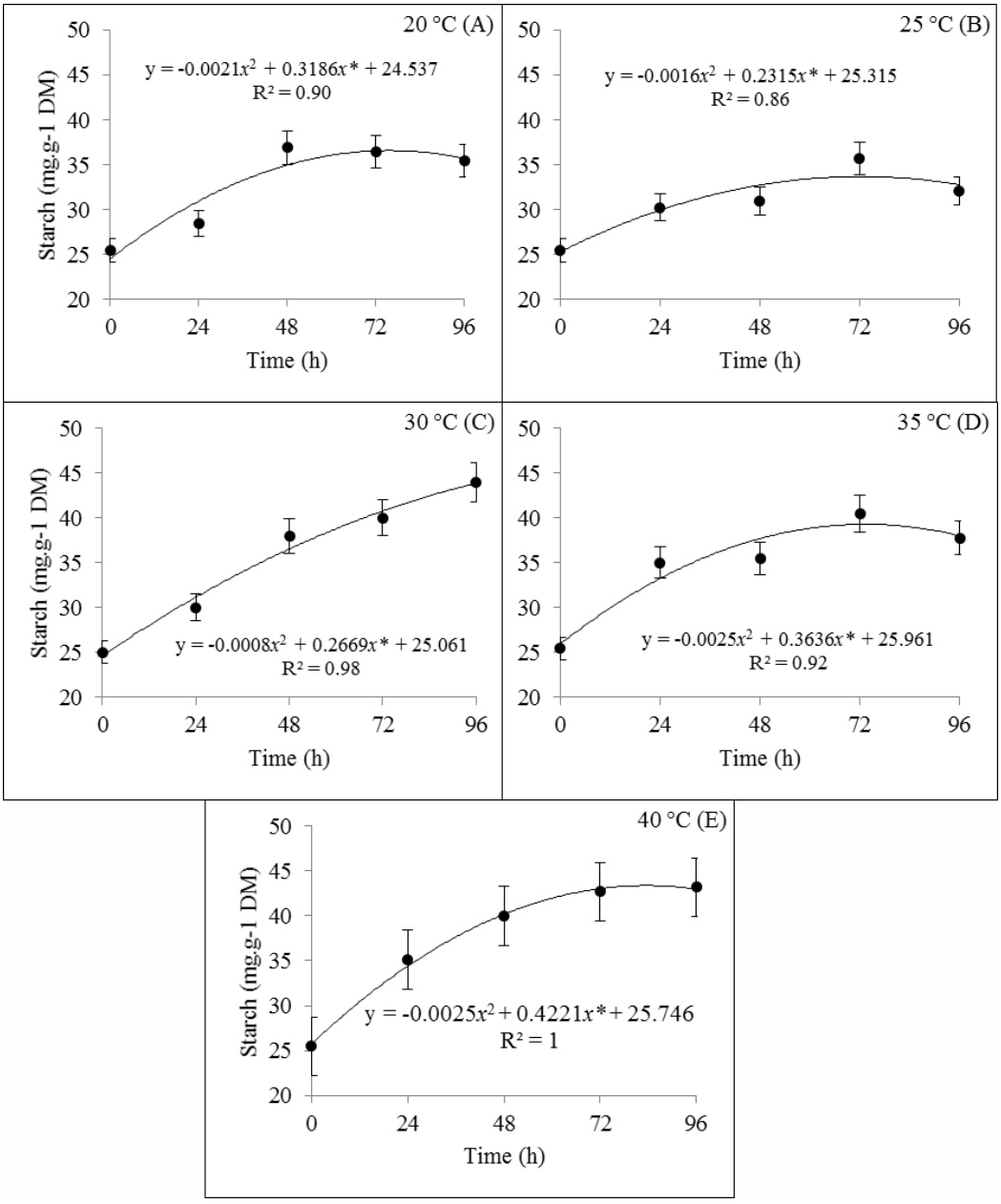

Figure 3. Starch mobilization in *Dalbergia spruceana* Benth. embryos during germination at different temperatures. DM, dry matter. An asterisk (*) indicates a significant difference at $p < 0.05$ by Tukey's test. Vertical bars represent the standard error of the mean (n = 5).

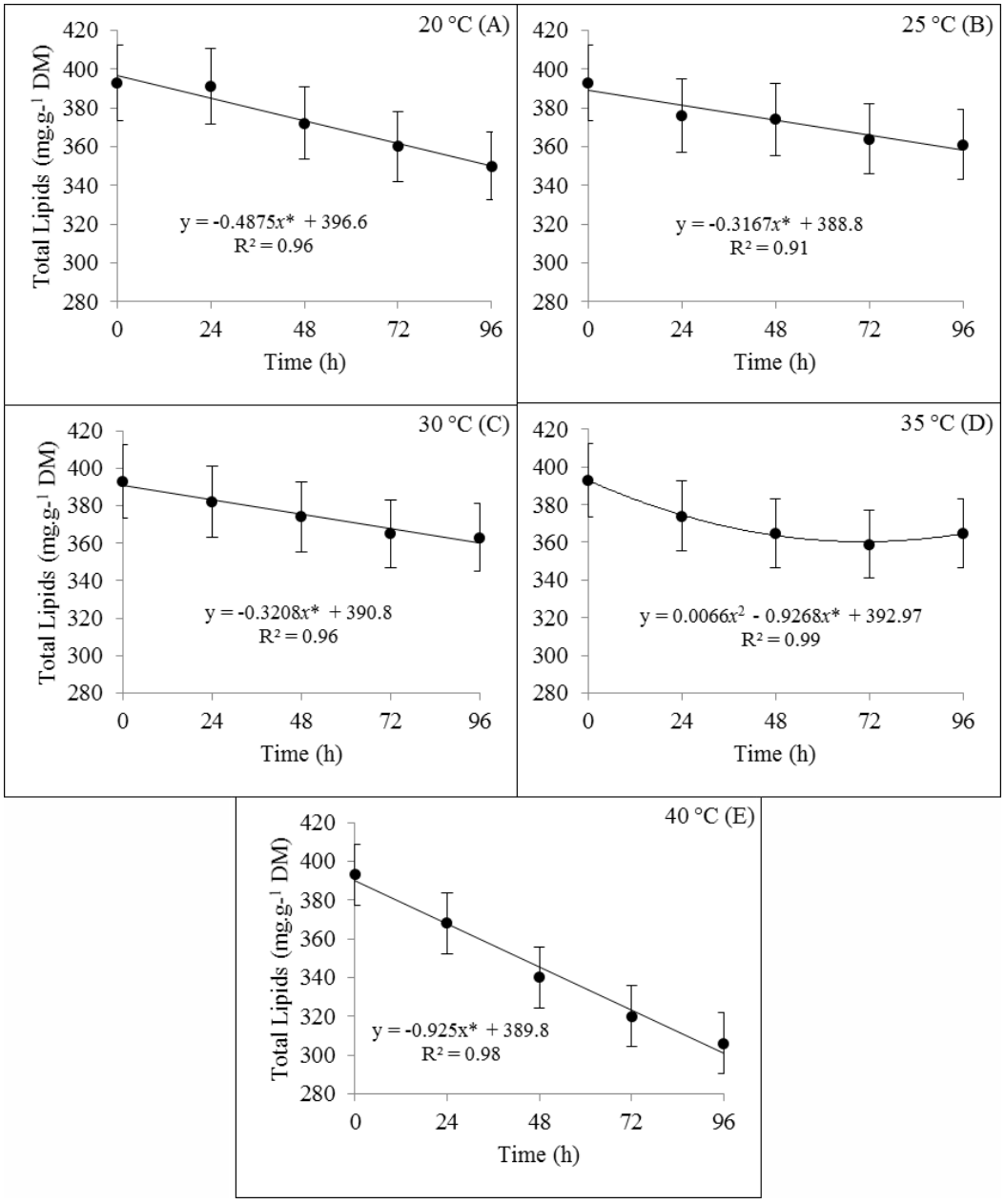

Figure 4. Lipid mobilization in *Dalbergia spruceana* Benth. embryos during germination at different temperatures. DM, dry matter. An asterisk (*) indicates a significant difference at $p < 0.05$ by Tukey's test. Vertical bars represent the standard error of the mean (n = 5).

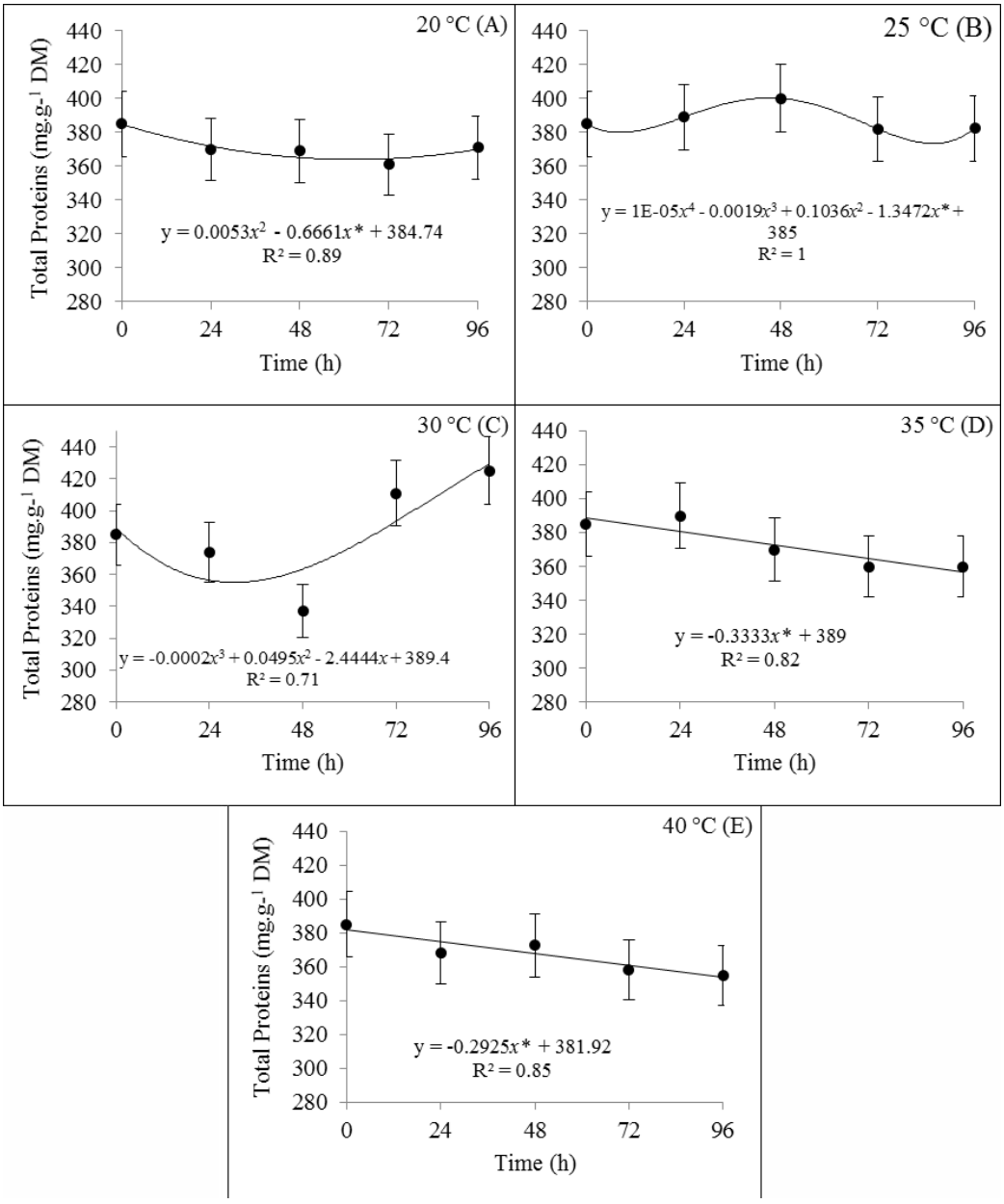

Figure 5. Protein mobilization in *Dalbergia spruceana* Benth. embryos during germination at different temperatures. DM, dry matter. An asterisk (*) indicates a significant difference at $p < 0.05$ by Tukey's test. Vertical bars represent the standard error of the mean (n = 5).

4. DISCUSSION

Dalbergia spruceana seeds were susceptible to low temperature during the initial imbibition phase, showing the lowest germination percentages and GSI at 20°C. It is likely that cell membrane reorganization was compromised at low temperatures, making the process slow and difficult (Tesfay et al., 2016).

On the other hand, final germination at 40 °C, could be explained because the respiratory efficiency of plant tissues is affected by enzyme denaturation and oxidative damage to membrane systems, limiting germination (Nievola et al., 2017; Santos et al., 2017). These alterations might have occurred in *D. spruceana* seeds subjected to high temperatures.

Mean germination time and germination speed indicate that, when kept a 25 and 30 °C, seeds the *D. spruceana* require lower time to germinate with greater speed de germination. The seeds incubated on these temperatures readily germinated within 48 h of imbibition, showing that metabolic rate increased at these temperatures, as also

observed in other tropical species (Santos et al., 2019; Reis et al., 2020; Felix et al., 2020a).

The rapid germination of *D. spruceana* seeds may be a strategy for the plant to establish itself in the environment as quickly as possible. As discussed by Duncan et al. (2019), the rapid germination rates, which suggests that rapid germination is particularly well suited to this environment and may be at a selective advantage over other seed germination strategies. From a physiological point of view, germination speed reflects the process of reserve mobilization. Storage reserves are used during metabolic reactivation when seed tissues are rapidly hydrated and mobilization during germination varies with the amount of reserves and the species (Zhao et al., 2018).

The predominance of lipids and proteins in *D. spruceana* seeds may be related to the rapid plant growth. The high lipid content of *D. spruceana* seeds may be considered an adaptive trait that allows seedling development under adverse environmental conditions, of temperature and humidity, given the high energy value of this component (Alencar et al., 2012).

Forest species have different mobilization strategies during germination that do not necessarily depend on the proportion of reserves. For instance, *Melanoxylon brauna* Schott seeds, have a higher lipid content, but carbohydrates and proteins are used first during germination (Ataíde et al., 2017). Similarly, in *Erythrina velutina* Willd. seeds, lipids are the second most abundant reserves but are not catabolized during germination (Felix et al., 2020a). In contrast, lipids play an important role in *Passiflora edulis* Sims (Tozzi and Takaki, 2011) and *Morinda citrifolia* L. (Paula et al., 2016) seeds; they are the main reserve compounds and are intensively mobilized during germination.

Carbohydrate metabolism may be associated with low-temperature stress (Xian et al., 2017). According to Xian et al. (2017), the increase in carbohydrate concentration under low temperatures may be a strategy to enhance osmotic potential and decrease the cooling point through expression of genes associated with sugar metabolism. In *D. spruceana*, because low temperatures reduced seed metabolism, observed through the lower germination speed at 20°C, soluble sugars were not rapidly used during germination, leading to reduced synthesis and germination percentage. In the leguminous tree *E. velutina*, reserve mobilization was more affected by low than high temperatures (Felix et al., 2020a). At low temperatures, the rates of enzymatic reactions and, consequently, cellular processes decrease.

Soluble sugars are essential for germination. Energy expenditure begins after imbibition and is maintained by sucrose reserves. These reserves are continuously consumed for ATP production in the embryo, the major energy consumer in seeds, because of the constant and rapid production of new cells (Lopes et al., 2013). At this stage, the demand for oxygen increases, altering cell carbohydrate levels. In *D. spruceana* seeds incubated between 25 and 30°C, soluble sugar levels were regulated to meet the energy demands of respiration and metabolic processes during germination. Species such *Moringa oleifera* L. (Tesfay et al., 2016), *M. brauna* (Ataíde et al., 2017) and *Ormosia coarctata* Jack. (Reis et al., 2020), with similar temperature requirements, also consumed sugar during germination. Optimal temperatures stimulate metabolic activity, promoting embryo development. The increase in soluble sugars content within the first 24 h of imbibition at 35°C may indicate changes in metabolic processes. However, such changes were not sufficient to negatively affect germination.

The metabolic pathways that generate energy and building blocks for radicle emergence are affected by high-temperature stress. The metabolic activity of *D. spruceana* seeds incubated at 40°C was compromised, leading to a reduction in soluble sugar availability. Such a result may be due to plasma membrane disruption and consequent leakage of metabolites into the extracellular environment (Santos et al., 2019). At high temperatures, DNA repair is also affected, resulting in loss of the ability to allocate energy reserves (Felix et al., 2020b). Therefore, high temperatures influence substrate availability for energy synthesis and promote degradation reactions.

Soluble sugars were likely made available to the *D. spruceana* embryo through metabolization of sucrose or raffinose family oligosaccharides, promoting embryo growth, as it happens in other Fabaceae species such as *Enterolobium contortisiliquum* (Vell) Morong.

The increase in starch contents in *D. spruceana* seeds is an indication of controlled use of reserves during rapid germination, probably as a strategy to maintain cellular osmotic balance and promote seedlitesng growth. According to Heldt (2005), glucose molecules are relatively unstable, as their aldehyde group can be spontaneously oxidized to carboxyl groups. Glucose can be polymerized to starch, which is osmotically inert and therefore maintan osmotic balance of the seed.

The increase in starch may have resulted from a surplus of hydrolysis products of lipid and protein. It is possible that the hydrolysis of primary reserves was more than sufficient for radicle emergence. Therefore, to maintain osmotic balance, excess metabolites were converted into a secondary reserve (in this case, transient starch), as also observed by Tozzi and Takaki (2011). The increase in starch levels observed in *D. spruceana* seeds indicates high availability of substrates for energy production during seedling development.

Lipids are not readily used by seeds. They must first be converted to sucrose by beta-oxidation; sucrose, in turn, is

converted to starch at high concentrations. Lipids are preferably stored in the form of triacylglycerols, which are nonpolar and can be stacked in a nearly anhydrous form. Their complete oxidation yields more than twice the energy afforded by protein or carbohydrate hydrolysis per unit volume (Bewley et al., 2013). This highly energetic molecule can therefore be used in the early stages of germination.

Protein metabolism initiates with imbibition, resulting in the production of free amino acids for biosynthesis and energy generation (Mazzottini-dos-Santos et al., 2017). In some cases, protein accumulation is associated with inter- and intracellular osmotic gradients caused by high cellular metabolic activity, indicating a shift toward seedling development (Bewley et al., 2013). This process may explain the increase in protein synthesis at 25 and 30°C in *D. spruceana*, with likely signaling the end of germination process and subsequent growth of seedlings at these temperatures. Protein synthesis was also observed during germination of *M. brauna* (Santos et al., 2019) and *Jatropha curcas* L. (Lopes et al., 2013) at optimum temperatures.

The mobilization pattern of *D. spruceana* seeds at different temperatures is similar to that of other legumes. In general, sub- and supra-optimal germination temperatures are detrimental to metabolic processes occurring during germination (Ataíde et al., 2017; Santos et al., 2019; Felix et al., 2020a).

In light of the future prospects of global warming, we believe that tropical species such as *D. spruceana*, which have a maximum temperature adaptation limit and, therefore, a narrow thermal tolerance range, are at risk. The negative impact of high temperature on germination metabolism may directly influence the establishment of *D. spruceana* in its natural habitat.

5. CONCLUSION

Reserve mobilization patterns in *D. spruceana* embryos were influenced by germination temperature. The utilization of hydrolysis products during embryo growth has greater efficiency in the optimal temperature range for germination, that is, between 25 and 35°C. At 20 and 40°C, soluble sugar mobilization is impaired, negatively affecting germination. Our results suggest that lipid and protein hydrolysis products are stored as transient starch. Soluble sugars are the main energy source for seed germination in *D. spruceana*.

SUBMISSION STATUS

Received: 21 Oct. 2022
Accepted: 28 Jun. 2023
Associate editor: José Carlos Arthur Junior

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Ely Simone Cajueiro Gurgel: Conceptualization (Equal), Investigation (Equal) Resources (Equal), Validation (Equal), Writing – original draft (Equal), Writing – review & editing(Equal).

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