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Distribution and chemical composition of crystals in wood of *Hevea brasiliensis* (Willd. ex Adr Jussieu) Muell. Arg.

Distribuição e composição química dos cristais na madeira de *Hevea brasiliensis* (Willd. ex Adr Jussieu) Muell. Arg.

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A B S T R A C T

In order to understand the intraspecific changes that occur in the rubber tree wood from São Paulo and Paraná (Brazil), the distribution and the chemical composition of crystals were investigated by morphological and chemical analyses using scanning electron microscopy and energy-dispersive *X-ray* spectrometry, respectively. Prismatic calcium oxalate crystals were frequently found in vessels and in parenchyma cells of both sites. The findings of this study suggest that crystals may be associated with the beginning of growth and also highlights the need for further investigations about soil nutrient to compare with crystal content found in *Hevea brasiliensis* wood.

Keywords

Calcium oxalate crystals, mineral inclusions, rubber tree, wood anatomy.

R E S U M O

A fim de compreender as mudanças intraespecíficas que ocorrem na madeira da seringueira de São Paulo e do Paraná (Brasil), a distribuição e a composição química dos cristais foram investigadas por meio de análises morfológicas e químicas por microscopia eletrônica de varredura e espectrometria de raios-X por dispersão de energia, respectivamente. Cristais de oxalato de cálcio foram frequentemente encontrados nos vasos e nas células do parênquima em ambos os locais. Os resultados deste estudo sugerem que os cristais podem estar associados ao início do crescimento, além disso, destaca-se a necessidade de realizar investigações sobre os nutrientes do solo para comparar com o conteúdo de cristal encontrado na madeira de *Hevea brasiliensis*.

Palavras-chave

Cristais de oxalato de cálcio, inclusões minerais, seringueira, anatomia da madeira.

Mineral inclusions occur in most plant families, in different plant tissues and cell types. Their composition, shape, size, and function are determined by a combination of genetic and environmental factors (Franceschi and Nakata, 2005). Crystal accumulation begins throughout the growing season and gradually becomes a significant anatomical component, suggesting that crystals may have an influence for systematics, physiology, and cell biology (Lersten and Horner, 2011).

Most recently, studying the wood anatomy of *Hevea brasiliensis*, Ramos *et al.* (2018) and Santos *et al.* (2019) also found mineral inclusions in wood cells. Native to the Amazon rainforest and member of the Euphorbiaceae family, *Hevea brasiliensis* (Willd. ex Adr Jussieu) Muell. Arg. is known as rubber tree and it is the most important commercial source for natural rubber production. The production cycle of latex last until 30 years (Rahman *et al.*, 2013) and after that, the rubber tree wood is frequently used in the energy sectors as a result of its low durability (Santos *et al.*, 2019).

As the chemical nature of crystals is rarely part of comparative anatomical studies, this work aims to describe not only the elemental composition but also their distribution in wood cells in order to know possible reasons for the presence of the crystals and the intraspecific changes that occur in the rubber tree wood from two different sites.

Eight trees of the Rubber Research Institute of Malaysia 600 (RRIM 600) clone of *Hevea brasiliensis* were used, from two commercial plantations at the end of the production cycle. Four 53-year-old trees were from Tabapuã, São Paulo (20°57′50″ S, 49°01′55″ W) and the other four 27-year-old trees were from Paranapoema, Paraná (22 ° 38′02″ S, 52 ° 05′52 ″ W), located in the southeastern and southern regions of Brazil, respectively.

Wood discs, from the middle of the trunk, of four trees were used from two sites, São Paulo (SP) and Paraná (PR). At the cross section, pith eccentricity was observed. Therefore, two types of wood were identified: opposite wood (OW) and tension wood (TW). Three samples with 1 × 1 × 1 cm dimensions were obtained from each type of wood corresponding to the following radial positions: next to the pith (P), an intermediate region (I), and next to the cambium (C). Transversal, tangential, and radial sections of 18 μm thickness were obtained for each sample by sliding microtome, and each of them was used to morphological and chemical analyses. Although there is no specific methodology to estimate the crystal frequency in woods, the quantifying vessel method per square millimeter was adopted in this study, according to IAWA (1989) recommendations. Thus, from each histological section, five images were obtained, and then the Image-Pro Plus® program was used to count crystals.

Scanning electron microscopy (SEM) images and energy-dispersive X-ray spectrometry (EDS) analysis were obtained for each histological section using FEI SEM Magellan 400 operating at 1 kV of acceleration potential and 0.13 pA of beam

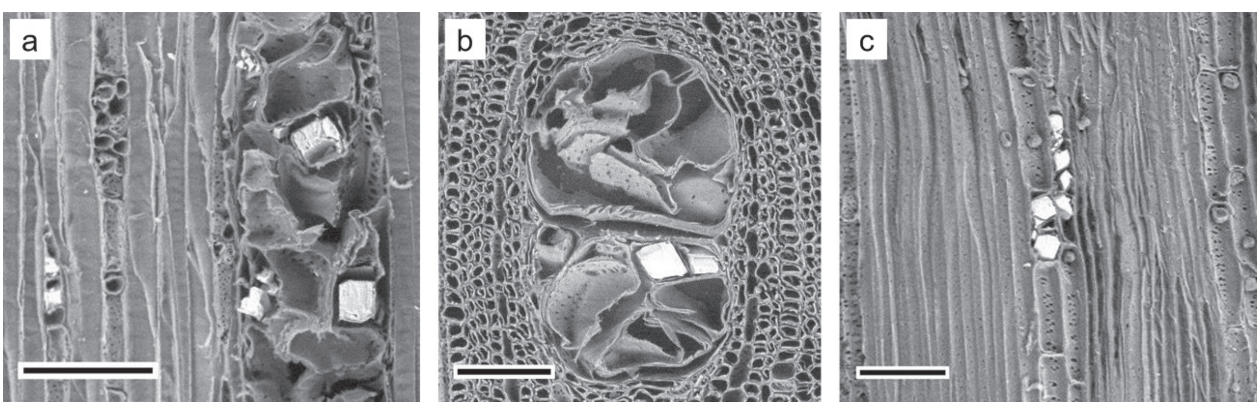

Figure 1 - SEM images illustrating the morphology and distribution of crystals in *Hevea brasiliensis* wood. a: tangential section, prismatic crystals in a vessel with tyloses and in radial parenchyma cells; b: cross section, crystals in a vessel with tylose; c: radial section, many crystals in axial parenchyma cells. Scale bar: 100μm.

current, to reduce the undesirable charging effect. Under these circumstances, no specific preparation was done, using dry histological sections directly for analysis. At least one crystal of each site was analyzed by spectrometry.

Many prismatic crystals were found in the parenchyma cells and the vessel elements of *Hevea brasiliensis* wood (Figure 1a-c). It was observed that most of the crystals found in the vessels close to the pith were associated with tylose (Figure 1a-b). Santos *et al.* (2019) also observed the same event in the structure of panel clones of *Hevea brasiliensis* but under the influence of two different canopy grafts and Ramos *et al*. (2018) observed in the structure of naturally grown rubber trees.

The frequency of occurrence of crystals (mean of crystals per mm^2) in the different types of wood (OW and TW) and in the different radial positions (Pith, Intermediate, and Cambium) for SP and PR sites were quantified (Figure 2a-b). Furthermore, to investigate the preliminary observations, the chemical composition of crystals was also analyzed (Figure 2c).

The trees established in the PR site had a higher frequency of crystals, regardless of the type of wood or radial variation (Figure 2a-b). Comparing the two types of wood, a higher frequency was observed in OW, in which the highest average value was above 116 (±28) crystals per mm^2, while in TW was 59 (±52) crystals per mm^2. At the SP site, the average values were lower when compared to the PR site. In OW, the highest average value was 26 (±18) crystals per mm^2, while in TW was 24 (±13) crystals per mm^2. It was also observed the frequency of the crystals in the radial variation of the *Hevea brasiliensis* wood, they were abundant in the region near to the pith and scarce in the region near to the cambium from both sites (Figure 2a-b).

The observations using EDS revealed the presence of calcium in crystals from all sites (SP e PR) and their composition of calcium oxalate is confirmed by these data (Figure 2c). However, crystals from PR site showed different elements as sodium, magnesium, aluminum, and silicon in their composition. The carbon relative quantity should not be considered, as well as the other element absolute quantification. In the other hand, the

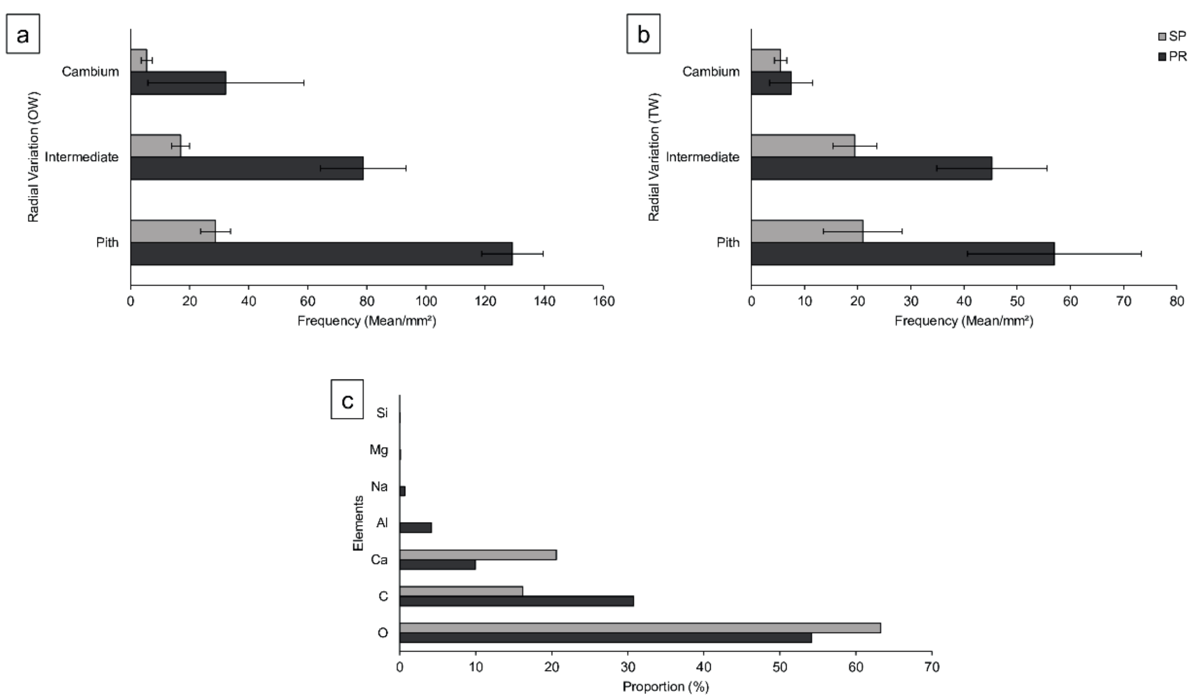

Figure 2 - Frequency of crystals (with mean standard error) per site, wood, and radial variation (a-b). EDS proportion of chemical elements found in the crystals of *Hevea brasiliensis* wood at São Paulo (SP) and Paraná (PR) sites (c). OW = Opposite Wood; TW = Tension Wood.

O/Ca ratio is a better and more reliable quantity from EDS analysis. In this case, it was measured that O/Ca_{SP} = 3.0 for SP site and O/Ca_{PR} = 5.4 for PR site, suggesting that the observed crystals are calcium oxalate of distinct water compositions.

The distribution of calcium oxalate crystals in plants is highly variable between species and their accumulation can vary throughout the growing season (Franceschi and Nakata, 2005; Lersten and Horner, 2011). Considering their biological significance, crystals can protect the plant from a range of environmental stresses, but some parameters as spatial, morphological and temporal of calcium oxalate crystal formation proposed that crystals can play a role in plant defense (Nakata, 2012). As the trees at the two sites come from a commercial plantation, a possible explanation for the significant presence of crystals, especially in the region close to the pith, may be related to defense against injury at the beginning of growth, once the species start yielding latex after 5 to 7 years of maturity, starting commercial exploitation (Rahman *et al.*, 2013). According to Ramos *et al*. (2018), the exploitation of latex affects the quantitative characteristics of xylem. When evaluating tapped and untapped native trees, the same authors observed an increasing width and height of ray cells in tapped rubber trees. Ray cells play several roles in the xylem, including mineral storage (Morris *et al*., 2016), which is in line with the present study, since most crystals were observed in those cells.

Another reason for the presence of calcium oxalate crystals is about calcium regulation in plants. Under conditions of calcium excess, the crystal appears to be involved in calcium sequestration reducing the apoplastic concentration around adjacent cells, but when the element becomes scarce in the environment, crystals are dissolved for growth and metabolic maintenance (Nakata, 2012). Crystals were frequently found in the trees from PR site. As these trees are younger than those from SP site, this event may be related to the initial wood growth. The calcium accumulation in a crystal form may be associated with a calcium storage for future needs. In contrast, a possible reason why trees from the SP site presented fewer crystals is that they reached maturity, so they may have accessed the calcium storage.

Another hypothesis about crystal presence in plants, cited by Franceschi and Nakata (2005), is tissue support or plant rigidity. It was observed that calcium oxalate crystals occur more in the OW than in the TW. Ramos *et al.* (2018) affirm that cultivated rubber trees are grafted plants, which can cause crown imbalance, leading to trunk displacement with the natural axis of the plant. Whereas the TW is formed where the tension forces are requested in the wood, the region with the lowest radial growth, defined as OW, may accumulate crystals in their cells to equalize the mechanical effort. It may also be related to the youngest phase of the growing period once calcium oxalate crystals were constantly found in the region near the pith.

Observing the distribution of crystals and their elemental composition in *H. brasiliensis* wood, it was concluded that: (1) Calcium oxalate crystals are present in different types of cells. (2) The elemental composition of crystals varies according to the site. (3) Younger rubber trees present a higher proportion of crystals. (4) The findings of this study suggest that crystals may be associated with the beginning of growth, considering they were frequently found close to the pith.

This study also highlights the need for further investigations about soil nutrient to compare with the crystal content found in *Hevea brasiliensis* wood. Such studies would provide a clearer insight into the reason for crystal presence.

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