DRUSES IN THE SECONDARY XYLEM OF *MANGIFERA INDICA* COLLECTED FROM COAL MINES, INDIA

A Gupta*, S Gupta & V Vishnuprasad

Wood Anatomy Discipline, Forest Research Institute, Dehradun, Uttarakhand 248006, India

*ankitabot125@gmail.com

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The study was undertaken to understand the influence of mining stress on the wood structure of tropical hardwood tree species. Dhanbad district in Jharkhand is the coal capital of India, and it is one of the most polluted areas of the world which makes it an ideal area of study to assess the mining stress on tropical hardwoods. Samples of mature wood were taken from the outer core of the sapwood of several hardwood tree species including seven samples of *Mangifera indica* along the fringes of the coal mines. Qualitative and quantitative features of the samples were studied. A conspicuous observation was made at the occurrence of druses in the ray parenchyma cells of a stressed *M. indic*a wood sample. The druses appeared as dense globular form, and the constituent units were like rose petals to glass flakes, representing a solid druse type. The occurrence of druses in *M. indica* has not been reported earlier. The presence of druses in a single sample of *M. indica,* out of seven, can be accounted to the adaptation of the individual plant to the local microclimatic conditions or side effect due to mining stress.

Keywords: Mining stress, systematic sampling, qualitative features, quantitative features, tropical hardwood

INTRODUCTION

Calcium oxalate crystals occur in more than 215 higher plant families (Franceschi & Horner 1980, Lersten & Horner 2006) including gymnosperms and angiosperms. In angiosperms, crystal formation is generally intracellular, forming inside the vacuoles of specialised cells called idioblast. Although the shape, size and number of crystals show variations among taxa, they are classified into five main groups based on their morphology: prism, druses, styloids, raphides and crystal sand (Webb 1999). Previous research have shown that crystal formation within the cell is under genetic control (Ilarslan et al. 2001). The formation of crystals with different habits seem to be associated with complex membranous systems within idioblast vacuoles that restrict environmental parameters where crystalogenesis takes place (Arnott et al. 1970). Therefore, crystal formation is not a random or haphazard process. The coordinated operation of proton pumps and ion channels to mobilise calcium and oxalate through the vacuole membrane is considered to underlie the whole process (Monje & Baran 2002). Most plants do not have well-developed excretory systems to dispose excess calcium. Higher plants appear to modulate differences between the natural abundance of environmental calcium and the very low levels required for cytosolic free calcium, by controlling the distribution of calcium and its compartmentalisation within the cell (Kinzel 1989). The most commonly proposed function of these crystals is the mechanism for removal of excess biologically active calcium when other mechanisms have become saturated (Volk et al. 2002). Through this mechanism, large amounts of excess calcium is precipitated as a highly insoluble salt of oxalate that is no longer osmotically or physiologically active (Zindler-Frank et al. 2001). Calcium oxalate crystals function primarily in sequestering excess calcium and/or act as a defense against herbivores (Arnott et al. 1970, Franceschi & Horner 1980).

The family Anacardiaceae consists of 77 genera and 700 species of trees, shrubs, woody climbers and very rarely, herbs. In India, the family is represented by 20 genera and 60 species of trees and 3 genera and 8 species of shrubs (Singh et al. 2000). Anacardiaceae is distributed in the tropical and subtropical regions of the world with a few extending into the temperate zones. The family is of considerable economic value. It produces edible fruits, gums and resins, tannins, dyes, drugs and several timbers of commercial importance. *Mangifera indica* wood is used in making light and heavy packing cases. However, its high grade timber and figured stock (curly grained) are suitable for furniture and cabinet work (Gupta & Agarwal 2008). The *M. indica* wood has growth rings, distinct, indistinct or even absent, diffuse porous woods, simple perforation plates, alternate polygonal intervessel pits, vessels 5–20 mm^{-2} tyloses common, non septate fibres with simple to minutely bordered pits, axial parenchyma ranges vasicentric, aliform, confluent, marginal type, all ray cells procumbent, prismatic crystals present in upright/square ray cells, procumbent ray cells and non-chambered axial parenchyma cells (Wheeler et al. 2004). Wood anatomical study on 22 genera of Anacardiaceae family from Southwest Pacific area showed the presence of abundant crystals, typically in square or upright marginal cells, usually solitary. Characteristically enlarged solitary crystal bearing cells occur sporadically in the marginal cells of *Odina, Pistacia* and *Pleiogynium,* and druses were observed in upright and procumbent cells of 2 species of *Rhus* (Dadswell & Ingle 1948).

Wood anatomy studies of Anacardiaceae from China were performed. A total of three tribes, Anacardieae, Spondieae and Rhoideae which included 11 genera and 22 species were studied. Prismatic crystals were present in all the genera and species belonging to the tribe Rhoideae, and druses were present for all the three species of *Rhus.* Prismatic crystals were present in all the genera and species of Spondieae tribe except *Choerospondias axillaria* and *Spondias lakonensis.* Similarly, prismatic crystals were present in all the species and genera of Anacardieae tribe except *M. indica* and *Buchanania microphylla* (Dong & Baas 1993). India ranks third as world coal producer from open cast mines. Jharia coal mines in Jharkhand, Dhanbad produces the best quality of coal in India. Places around Dhanbad are rich in coalfields consisting of 112 coal mines which makes the city the coal capital of India. Mining started in 1894 in Dhanbad and since then mines and minerals have been the main source of economy in Jharkhand. However, the air pollutants reduce air quality and this ultimately affects the flora and fauna in and around mining areas (Chaudhari & Gazghate 2000). The major air pollutants produced by opencast mining are suspended particulate matter and respirable particulate matter (Sinha & Banerjee 1997).

Coal is the primary source of energy. The development of industries has a downstream effect on the demand of coal. The utilisation of coal is limited by environmental disruption, including deterioration of air quality due to emission of particulate matter and other gaseous pollutants from various mining operations, which have adverse environmental impacts. In the opencast mining, the volume and variety of air borne dust particles in the ambient air is rapidly increasing leading to degradation of natural resources and destruction of habitat, ultimately causing threat to biodiversity (Ghose & Majee 2001). It destroys the forests as well as ground vegetation and alters the physico-chemical and biological properties of the soil. Regular blasting disturbs the soil, underground flora and fauna and contributes to immense gaseous pollutants. The mine soils feature salinity, acidity, poor water holding capacity, inadequate supply of plant nutrients and accelerated rate of soil erosion (Jha & Singh 1992). Biochemical, morphological and anatomical responses brought about by gaseous pollutants, either under natural or experimental conditions, have been reported earlier (Mudd & Kozlowski 1975).

Early literature on the wood anatomy of Anacardiaceae family has been provided by Metcalfe & Chalke (1950). For some species, often from restricted areas, several wood anatomical descriptions have been published (Dadswell & Ingle 1948, Chattaway 1956, Grundwag & Werker 1976, Baas & Schweingruber 1987, Chauhan & Dayal 1990, Dong & Bass 1993, Carlquist 2001). Studies on wood anatomy of *Mangifera* species have been published (Dadswell & Ingle 1948, Corothie 1960, Ahmad & Khan 1986, Dong & Bass 1993, Wheeler et al. 2004, Gupta & Iqbal 2004, Rajput & Rao 2007, Gupta & Agarwal 2008, Dave & Rao 2008, Padhiar & Albert 2012).

METHODOLOGY

Sampling and collection

With the aim to study the effects of coal mining stress on wood anatomical features of tropical hardwood species, wood samples were collected following a statistical systematic sampling plan. Three points (representing each position) represented by a line (L) on the perimeter of the mine were chosen such that the perpendicular from the points were taken up to the fringes of the mines. Three quadrats (Q) of size 20 m × 20 m on each line were laid at a distance of 800–1000 m from each other. This plan was triplicated for three different coal mines. Mature tropical trees (5–8) were randomly selected from each quadrat to study the wood samples. Mature wood samples were collected from 17 tropical wood species with 110 samples of different hardwood species across three different coal mines, out of which 7 wood samples belonged to *M. indica*. Mature wood samples, each of 2–3 mm thickness depth in radial view, were collected from the sapwood of the main trunk of different tree species using increment borer instrument. For each tree from which the wood sample was collected, the GPS coordinates, circumference, diameter, height, vegetative characters and health condition of the trees were noted. Plant materials of respective tree species were collected for the herbarium, and identified by the Botanical Survey of India, Dehradun, India.

Sectioning

The wood samples of seven stressed *M. indica* were soaked for 24 hours to soften them. Transverse and longitudinal sections of 12–20 µm thickness were cut on a sliding microtome and stained with safranine. After dehydration in ethanol-xylene series, the sections were mounted in dibutyl phthalate xylene (DPX).

Data Collection

Quantitative and qualitative data for the seven *M. indica* stressed samples were studied under compound light microscope. Microstructure study was according to the terminology by the International Association of Wood Anatomists (IAWA 1989). Sections were studied for their microstructure along with the types and locations of crystals and druses, if present. Diameters of the cell inclusions were measured. The photomicrographs of the sections were observed using a microscope with Axio Vision Rel 4.0 software.

RESULTS

While studying the wood anatomical parameters of different wood samples collected to assess the effect of mining stress, a striking observation was made in one of the stressed sample of *M. indica*, collected from Jharia coal mine area, Dhanbad, Jharkhand, India. The tree was approximately 3–35 years of age, with 98 cm circumference and 12.5 m height. The GPS coordinates were latitude 23° 44' N and longitude 86° 26' E. Screening of slides under the microscope established the occurrences of druses in *M. indica*. Druse crystals have a variety of sizes, types, globular forms and constituent units (Chairiyah et al. 2013). With reference to this, the druses found in the current study were medium sized with diameter between 15–20 µm, had dense globular form and the constituent units were like rose petals to glass flakes, representing a solid druse type.

In most places of the radial tangential section, the druses were present in upright ray parenchyma cells, and at very few places, they were present in the procumbent ray cells (Figure 1a). Chambering was observed in the upright cells, forming 2–3 chambers, occupied by solitary druse (Figures 1b and 1c). Few procumbent cells, underlined by a single margin of upright cells, exhibited the presence of druses, though less frequent than the upright cells. In the ray procumbent cells, chambering was also observed with solitary druse in each chamber (Figure 1d). At few instances, two different cell inclusions i.e. druse and a crystal were seen to co-exist in a single procumbent ray cell (Figure 1c), as well as in the chambered upright cell (Figure 1f), whereas in some procumbent cells, a druse is solitarily occupied (Figure 1d). The diameter of the druses were 15–20 µm and crystals were 15–38 µm. Some crystals were bigger, up to 38 µm. Among the seven different radial longitudinal section (RLS) of *M. indica* samples, sized 0.30 mm × 0.11 mm (× 4), 13–25 druses were observed, whereas 16–25 crystals were present on average.

DISCUSSION

The presence of druses in *M. indica* has not been reported (Metcalfe & Chalk 1983). Druses are present only in *Rhus* species in the Anacardiaceae family (Carlquist 2001, Dong & Baas 1993, Dadswell & Ingell 1948). Pearson & Brown (1932) has not mentioned the presence of druses in *M. indica*, though the presence of crystals was mentioned. The Inside Wood database also does not report the presence of druses in *M. indica*. Gupta & Agarwal (2008), while studying the members of Anacardiaceae family, found the

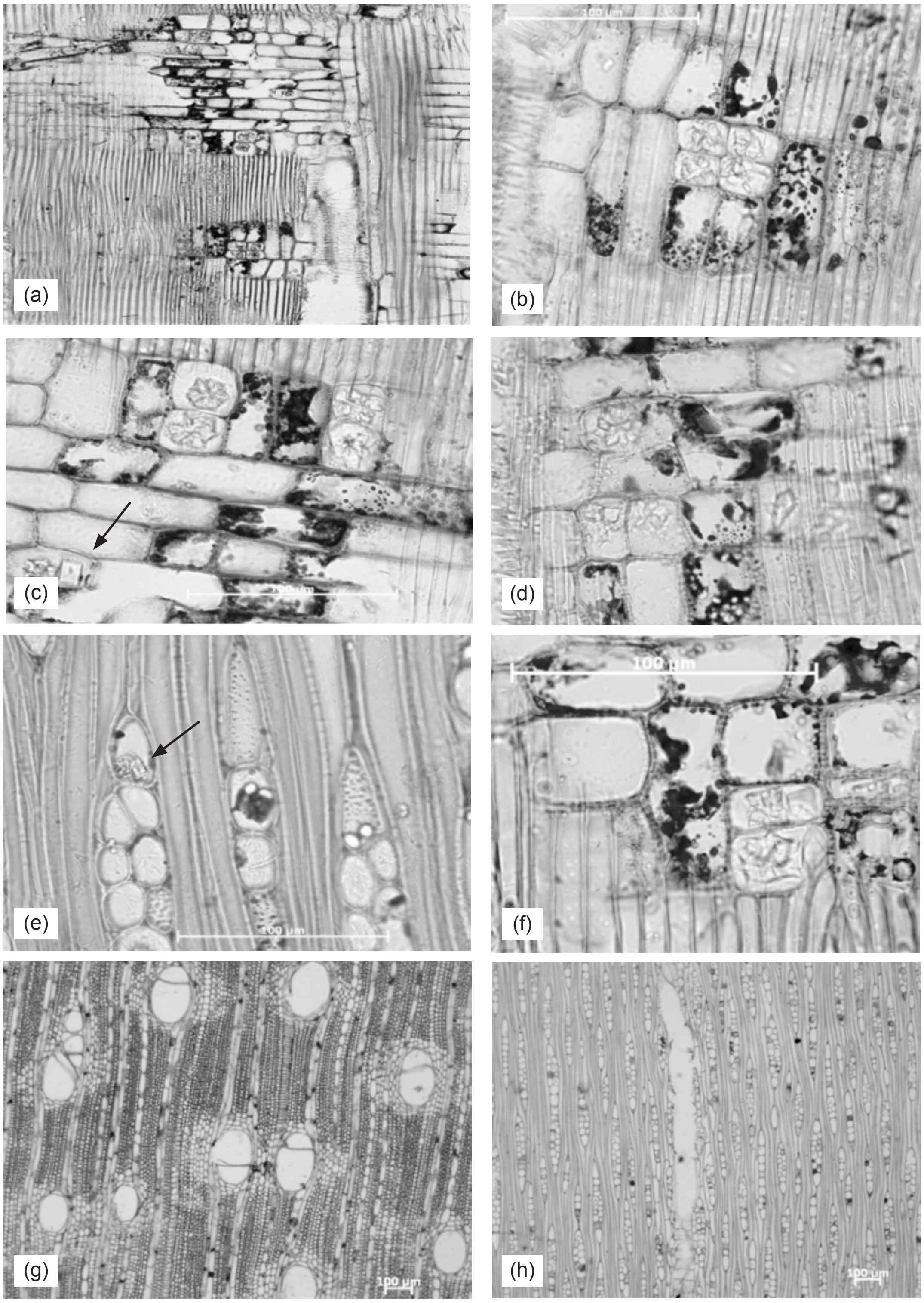

Figure 1 (a) Radial longitudinal section (RLS) of *Mangifera indica,* druses in chambered ray parenchyma cells (×10), (b) chambering in upright ray parenchyma cells, each upright cell is divided to form three chambers where inner two chambers were occupied by solitary druse (×40), (c) druses embedded in the chambers of the upright cells (each divided to form two chambers) of ray parenchyma cell from a different location in the RLS of *M. indica,* the arrow represents the co-existence of a druse and a crystal in a single ray procumbent cell (×40), (d) a portion of ray procumbent cells showing the presence of solitary druse in a single ray procumbent cell as well chambered procumbent cell (×40), (e) tangential longitudinal section (TLS) of *M. indica,* druse in the upright ray parenchyma cell (×40), (f) co-existence of a druse and a crystal in chambered upright ray parenchyma cell (×40), (g) transverse section of *M. indica,* (h) TLS of *M. indica* (×10)

presence of druses in *Semicarpus khurjii* but not in *M. indica*. Hence, the present study forms the first report to feature the presence of druses in *M. indica*. Dong & Baas (1993) have mentioned the absence of prismatic crystals in *M. indica,* but the present study reports the presence of abundant prismatic crystals in all the seven wood samples of *M. indica*.

The evidence of the presence of druses in one sample of *M. indica* can be accounted to the microclimatic conditions/side effect due to mining stress. The microclimatic conditions include intracellular regulation of pH, calcium ions, gravity perception, mechanical support and plant defence (Monje & Baran 2002), which can lead to the formation of druses at the stressed site. Marcati & Veronica (2005) have indicated the abundance of crystals during water deficit condition. The area of collection gave an impression of a drought habitat due to severe erosion of soil and reduction in fertility, polluted waters, drained underground water reserves, alteration of landscape or damaged roads. The concept of calcium signatures is well established and the tight control of the temporal and spatial characteristics of cytosolic calcium alterations is responsible for the specificity of various cellular responses, in particular to environment-induced stress, in this case, mining stress (Hong-bo et al. 2008). Elevated calcium levels helps to recover from toxicity and damages from environmental stress (mining stress) through calcium signalling. Druse crystal formation was found to be dynamic and responsive to fluctuations in calcium levels. When calcium levels were high, druse crystal size and number rapidly increased. When calcium was limited, druse crystal size and number decreased, presumably freeing up the calcium for utilisation by the plant (Faheed et al. 2013). Thus, different crystal morphologies and distributions could accommodate different rates of ion removal within and among tissues. Crystals have evolved with respect to environmental niches occupied by particular plant species and in concert with physiological parameters affecting calcium uptake and oxalate synthesis (Webb 1999). Isodiametric druses to clustered crystals were observed in large idioblasts in the axial parenchyma in all specimens of *Terminallia catappa* except cultivated ones, which hints greater tendency of druse formation in stressed sites than cultivated sites (van Vliet 1979). The xylem of trees affected by air pollution was characterised by the presence of crystalliferous fibres, developed in latewood, whereas the formation of such fibres was not found in the normal trees (Rajput et al. 2008). There is a possible contribution of calcium oxalate formation in sequestering and tolerance of at least some toxic metals in stressful sites (Mazen et al. 2002, Lutts et al. 2004). The histological variations in the wood of *M. indica* in response to coal smoke pollution exhibits ecotypic significance (Gupta & Iqbal 2004).

CONCLUSION

In conclusion, the present study suggested the presence of druses in individual *M. indica* as a result of adaptation to microsite/site–specific severe stressful conditions.

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