THE LOCATION OF STARCH IN RUBBERWOOD

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Abstract: Rubberwood (*Hevea brasiliensis*) contains substantial amount of starch, especially in the sapwood portion of the tree. This has been proven by iodine test whereby sapwood turns clarker blue in colour but heartwood becomes lighter blue. The exact location of starch in rubberwood has not been studied in detail especially at the microscopic level of wood cells. This paper is an attempt to discover the nature and location of starch with the aid of light transmission microscope and scanning electron microscope (SEM). Interestingly, starch was observed in ray parenchyma and wood parenchyma cells. All wood parenchyma cells contain starch but only the upright cells of the ray parenchyma reveal the presence of starch. The nature of starch was either spherical or pear-like and pea-like shapes.

Keywords: Rubberwood, Starch, Ray parenchyma, Wood parenchyma, Microscope

INTRODUCTION

Starch can be found in the sapwood of certain hardwood species but not common in softwood species. Although starch grains may occur in fibers, they are more common in longitudinal parenchyma and ray cells and their shapes are frequently spherical [2]. Starch is necessary in the diet of powder-post beetles and its absence could make timber immune to Lyctids attack [8].

The presence of starch in rubberwood (*Hevea brasiliensis*) is associated with the blue-staining fungi. This is easily seen when rubberwood logs are left to rot in the rubber plantation. The cross section of the log turns blue in colour upon exposure and portion near the bark, that is sapwood exhibited remarkable colour change compared to that of heartwood.

The nature and location of starch in rubberwood at the microscopic level has not been studied in detail. It is therefore the objective of this study to reveal the findings regarding the presence of starch with the aid of transmission and scanning electron microscopes.

MATERIALS AND METHODS

The blocks of rubberwood $(1 \text{ cm } x \ 1 \text{ cm } x \ 2 \text{ cm})$ were cut by chisel and razor blade and later softened by boiling them in water until they sank [4]. The softened blocks were stored in glycerol-alcohol (1:1 by volume).

Rubberwood blocks were sectioned using a sledge microtome while keeping them wet with alcohol [6]. Selected cross, radial and tangential sections were treated with a solution of iodine in potassium iodide for starch determination.

For observation under scanning electron microscope (SEM), small rubberwood blocks ca. 1 cm3 were cut from the wood blocks with faces carefully oriented in cross, radial longitudinal and tangential longitudinal planes. This was then mounted on aluminium stubs and later placed in a vacuum coating apparatus. The stubs were rotated while a layer of gold 50 - 70 nm thick was deposited over the whole specimen [1]. Finally, the rubberwood specimens were examined in a Cambridge Instrument Co. 'Stereoscan' S600 scanning electron microscope.

RESULTS AND DISCUSSIONS

When thin cross, radial and tangential sections of rubberwood sapwood were treated with a solution of iodine in potassium iodide, certain regions became almost black with a slight tinge of purple due presumably to the formation of the intensely coloured starch-iodine complex [9].

The types of starch commonly found in natural products are amylase and amylopectin [9]. Amylose in low concentration reacts with iodine to give an intense blue solution: in more concentrated solution a blue-black precipitate is formed. Amylopectin reacts with iodine to give a reddish-purple colour. When the cross section is viewed under the transmission optical microscope (Figure 1), some of the rays (Ra) appear dark but other rays (Rb) are nearly colourless which means that not all ray cells contain starch. Both the apotracheal (APa) and the paratracheal parenchyma (PPa) are relatively dark. The pores (Po) and the fibres (F) are not stained by the iodine treatment. The radial section photograph (Figure 2), which for comparison should be rotated through 90°, also shows darker areas in the vertical parenchyma (VPa) and in the upright cells (Uc) of the rays but again the fibres (F) and the procumbent cells (Pc) of the rays are not stained. A similar pattern appears in the tangential section (Figure 3) which confirm that starch is present in the vertical parenchyma (VPa) and in the upright cells (Uc) of the rays but is not found in the fibres (F) and in the procumbent cells (Pc) of the rays.

Electron micrographs (Figure 4) provide additional evidence for the presence of starch. The use of SEM in the study of wood anatomy [10] dates form 1968 and has been applied to small blocks of wood cut true to the three planes of orientation [3]. One of the advantages of SEM lies in the simplicity of specimen preparation because the solid surface of the object can be observed directly. Accordingly, observation of starch granules was done using rubberwood blocks instead of ultra-thin sections as in the case of transmission electron microscopy (TEM). Starch granules of various shapes are visible in the vessels (or pores) (Plate 5a), in the apotrachael parenchyma (Figure 5), in the upright cells of the rays (Figure 6), and in the vertical parenchyma (Figure 7). Starch grains have previously been reported [1] in axial parenchyma, rays, and fibres of certain hardwoods but their precense in rubberwood has been recorded. The seeds of the rubberwood tree [5] contain starch which was identified as amylose.



Figure 1: Cross section of rubberwood slide sample treated with iodine



Figure 2: Radial section of rubberwood slide sample treated with iodine



Figure 3: Tangential section of rubberwood slide sample treated with iodine



Figure 4: Starch in ray parenchyma cell



Figure 5: Starch in wood parenchyma cell



Figure 6: Starch in upright cell of ray parenchyma



Figure 7: Starch in almost all cells of wood parenchyma

CONCLUSION

Observation of slide specimens by light transmission microscope and ultra-cellular level of rubberwood by scanning electron microscope (SEM) draw the following conclusion; sapwood portion of rubberwood contains starch only in certain cells namely ray parenvhyma and wood parenchyma. The upright cells of the ray parenchyma possess starch but not the procumbent cells. On the other hand, almost all cells of the wood parenchyma contain starch. The starch appears as pear-like and spherical shape in the ray parenchyma and pea-like shape in the wood parenchyma cells.

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