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RELATIONSHIP OF INCREASED PEROXIDASE ACTIVITY AND DECREASED CYTOKININ CONTENT IN TPD AFFECTED *HEVEA* TREES

R. KRISHNAKUMAR, S. SREELATHA, JAYASREE GOPALAKRISHNAN, JAMES JACOB and M.R. SETHURAJ

Rubber Research Institute of India
Kottayam - 686 009
Kerala

The tans-zeatin riboside (t-ZR) levels, peroxidase activity and phenol content in the bark tissues of normal and TPD affected *Hevea* trees of clone RRII 105 were analysed. The cytokinin from each bark tissue samples was extracted. The partially purified extracts were used to determine the t-ZR content in the bark tissue through direct ELISA using polyclonal antibodies raised against t-ZR-BSA conjugate. The normal plants showed significantly higher t-ZR content than the TPD affected plants. The higher t-ZR content in normal plants was negatively correlated with their peroxidase activity and phenol content. The biological significance of these negative correlation is discussed vis-a-vis biotic stress and free radical scavenging.

INTRODUCTION

One of the most serious problems affecting the productivity of rubber plantations is tapping panel dryness (TPD) which is more often seen in the high yielding clones of *Hevea*. The incidence of TPD is estimated to cause 15-20% decrease in yield per year (Commerce *et al.*, 1989). A large number of biochemical and physiological investigations have been conducted to study and describe this disorder (Prematillaka *et al.*, 1985; Gomez *et al.*, 1990 and Dian *et al.*, 1995). It is more or less concluded that no pathogens are associated with TPD and it is now considered as a physiological disorder. The involvement of plant growth regulators in TPD is not clearly understood yet. It is essential to study the role of growth regulators along with other biochemical components, particularly those involved in the scavenging of free radicals which are over produced during the physiological disorders. Many studies have revealed that under stress condition, plants generally increase the production of free radical scavenging enzymes (Siegel, 1993) and secondary metabolites like phenols (Gupta *et al.*, 1995). Under oxidative stress, peroxidase activity often increases and one of the principal roles of peroxidase appears to be cellular protection from oxidative stress in plants (Siegel, 1993). Apart from this, peroxidase also promotes a large variety of biological reactions. This enzyme system is reported to catalyse the biosynthesis of ethylene (Machackova and Zmrhal, 1981) and degradation of indole-3-acetic acid (Grambow and Langenbeck-Schwich, 1983). However, peroxidase enzyme activity was reported to be inhibited by exogenous application of cytokinins in rose plants (Zieslin and Ben-Zaken, 1992). Information on the role of peroxidase activity in relation to the cytokinin content is limited in plants, and more so in the case of TPD affected *Hevea* plants. Therefore, a study was conducted

with an objective to evaluate the relationship between endogenous trans-zeatin riboside (t-ZR) content and the peroxidase activity, and phenol contents in normal and TPD affected bark tissues of *Hevea brasiliensis*.

MATERIALS AND METHODS

Eighteen year old plantation of *Hevea brasiliensis* (clone RRII 105) from the Central Experimental Station of RRII at Chethackal was selected for this study. All agronomic practices and plant protection were undertaken according to RRII recommendations. These trees were subjected to regular tapping, thrice in a week, throughout the year for the past two years. TPD affected and normal trees were identified by tapping observations during two months time prior to the bark sample collection. Bark tissues from the normal (n = 15) and TPD affected trees (n = 15) were removed carefully from the tapping panel. The bark samples were collected on ice and transported to the laboratory and stored at -60°C. Soft bark tissues were excised and used for the biochemical analyses.

Cytokinin (t-ZR) extraction and estimation

The cytokinin extraction medium contained 80% methanol with butylated hydroxytolune (BHT) as antioxidant (10 mg/l). The extraction was done from bark sample (10 g) in duplicates after Weiler (1980). The crude extracts were partially purified by polyvinyl polypyrolidone (PVPP) columns. The aqueous extracts were lyophilised and reconstituted with 2 ml of 20mM tris buffered saline (pH 7.5). The trans-zeatin riboside (t-ZR) and Bovin Serum Albumin (BSA) used in this experiment were purchased from Sigma Chemicals, USA. The t-ZR was conjugated to a hapten (Wrlnger and Beiser, 1964), to raise the polyclonal antibodies in 12-16 weeks old Newzealand rabbits. The t-ZR contents in the bark tissue samples were analysed by direct enzyme linked immunosorbant assay (ELISA) using t-ZR as the standard (Vonk *et al.*, 1986). The bark cytokinin content was expressed as pmol/unit fresh weight of bark tissue.

Peroxidase enzyme activity

Peroxidase enzyme activity in bark sample was measured after the method of Shannon *et al.* (1996). The peroxidase extraction was done with 0.1M phosphate buffer (pH 6.5). The extract was used for the assay and the enzyme activity was calculated and expressed as change in activity/min/mg protein. The protein content in the extracts was determined according to Lowry *et al.* (1951).

Phenol content

The total phenols of the bark tissues were extracted in 80% ethanol and estimated following the protocol developed by Swain and Hills (1959). The phenol content in the bark tissue was expressed as mg/g fresh weight of the tissue.

The individual biochemical parameters analysed in normal and TPD affected trees were compared using t-test. The relationships between the cytokinin and the peroxidase activity and phenol contents were regressed using the method of least sum of squares.

RESULTS AND DISCUSSION

The analyses showed that TPD affected trees have comparatively lower levels of t-ZR content in the bark than the normal trees (Fig. 1A). The difference between the endogenous t-ZR levels in normal and TPD affected plants was found to be statistically significant ($p<0.001$). Although several studies have been carried out to study and describe the development of TPD in *Hevea*, most of them are mainly limited to the latex biochemistry (Bealing *et al.*, 1972; Commerce *et al.*, 1989; Dian *et al.*, 1995). No reports are available on the role of phytohormones present in the bark in relation to TPD. Cytokinins are essential in a plant tissue to maintain its juvenility and to retard senescence.

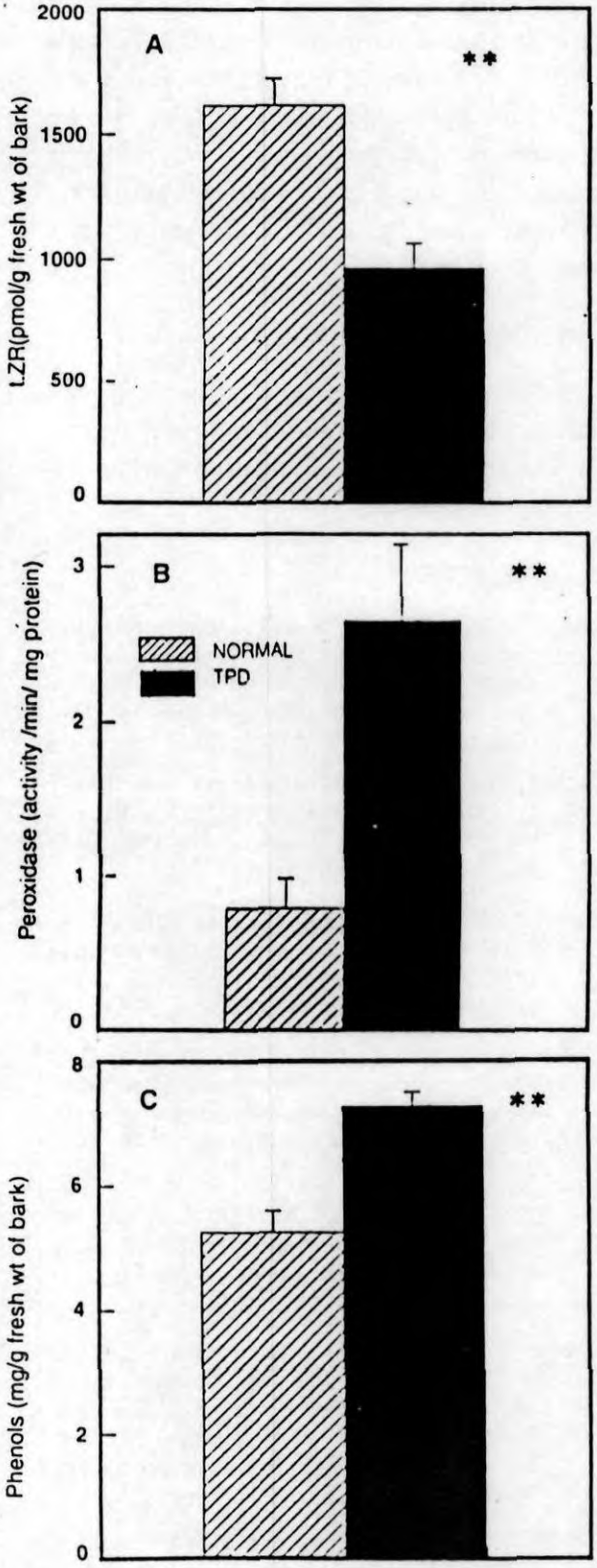

Fig. 1. Concentration of t-ZR(A), phenols (C) and activity of Peroxidase (B) in normal and TPD affected bark tissues. **Significant at P = 0.01

Therefore, maintenance of high content of t-ZR may be essential for the active metabolism of normal bark tissues.

The biochemical analyses of the bark samples revealed that the TPD affected plants contained significantly higher levels of peroxidase activity (Fig. 1B) and increased phenol content (Fig. 1C). The higher peroxidase activity in TPD affected trees may be related to large production of $O_2^{\cdot}$ due to distorted physiology in TPD affected tissues leading to oxidative stress (Siegal, 1993).

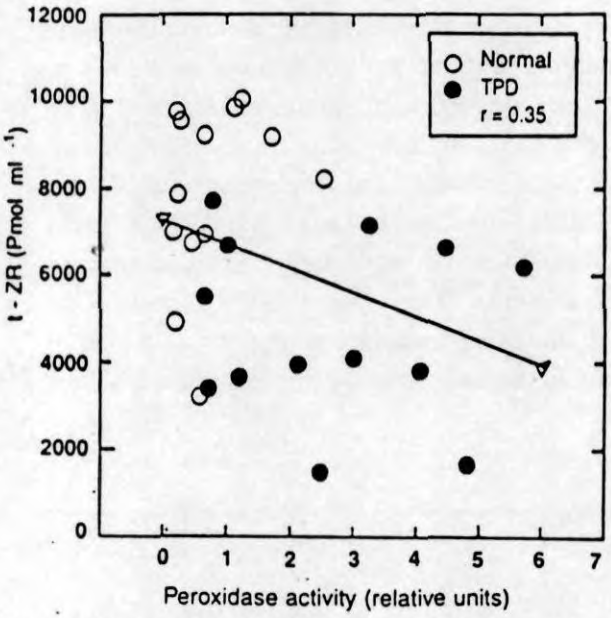

Fig. 2. Relationship between t-ZR and peroxidase activity in the soft bark tissues of *Hevea brasiliensis*

The enhanced phenol content in the TPD affected plants may be the result of biochemical regulation of secondary metabolites due to the biotic stress induced by TPD. Accumulation of phenolic compounds has been noticed in plants under biotic stress caused by pests and diseases (Gupta *et al.*, 1995).

The bark t-ZR content had an inverse relationship with peroxidase activity (Fig. 2) and phenol content (Fig. 3). But the cause-effect relations cannot be elucidated from the present

study. However, certain studies have indicated the role of peroxidases and phenols in the regulation of some phytohormones such as indol-3-acetic acid (IAA) and ethylene. For example, peroxidases are capable of catalysing the conversion of aminocyclopropane carboxylic acid (ACC) to ethylene (Machackova and Zmrhal, 1981; Rohwer and Mader, 1981). Grambow and Langenbeck-Schwich (1983) reported that the peroxidases catalyse the degradation of IAA resulting in the formation of indol-3-methanol (IM) in the presence of phenolic compounds. IM is the first detectable product which is further oxidised to the corresponding aldehyde (Grambow, 1986).

In normal tissues the cytokinin levels are maintained either by synthesis, degradation or by interconversion. Cytokinin is reported to be degraded through the activity of cytokinin oxidase that removes the isopentenyl side chain, yielding the adenine or adenosine form (Chatfield and Armstrong, 1986; Letham and Palni, 1983). The inverse relationship of t-ZR content with peroxidase activity or with phenol content alone is inadequate to suggest whether they catalyse any of the metabolic reactions to maintain a low tissue cytokinin level in TPD affected bark tissues. More investigations are needed to suggest the cause-effect relationships of peroxidase activity and phenol content, if any, towards the changes in the endogenous cytokinin levels in healthy and TPD affected bark tissues.

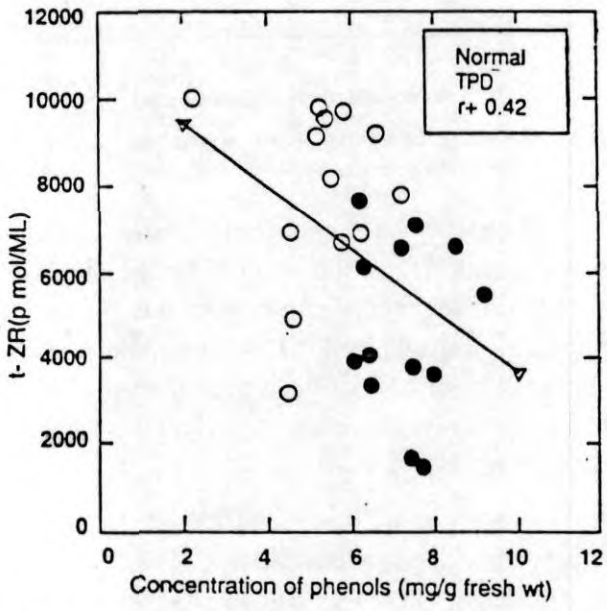

Fig. 3. Relationship between t-ZR and phenols in the soft brak tissues of *Hevea brasiliensis*

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