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INFLUENCE OF TPD ON CYTOKININ LEVEL IN *HEVEA* BARK

Cytokinin, a major group of phytohormones, are found in tissues with active metabolism. Their mode of action includes interaction with t-RNA metabolism, effects on membrane permeability and localized induction of metabolic sinks (Letham and Palni, 1983). Cytokinins can act as an antisenescence phytohormone by direct scavenging of pre-existing free radicals and/or by incipient prevention of free radical formation (Leshem, 1984). Several studies on the biochemistry of tapping panel dryness (TPD) are available, but the role of plant growth regulators in TPD is unknown. There are indications that the metabolic breakdown in TPD affected tissues may be related to damages inflicted to cellular components by free radicals and active oxygen species. Chrestin (1985) showed an abnormal production of toxic oxygen molecules in *Hevea* trees overstimulated with ethrel and also reported that over-exploitation of rubber trees with ethrel often leads to TPD. It may be noted that ethrel, upon hydrolysis, generates ethylene (Audley *et al.*, 1978) which is known to induce senescence possibly by over production of active oxygen species (Chrestin, 1985), but cytokinins can resist this (Leshem, 1984). Therefore, a study was conducted with an objective to evaluate the levels of endogenous trans-zeatin riboside (t-ZR), a dominant group of cytokinin seen in higher plants, in the bark tissues of healthy and TPD affected *Hevea* trees.

Eighteen year old *Hevea brasiliensis* trees of clone RRII 105 from the Central Experimental Station of the Rubber Research Institute of India at Chethackal, Kerala were selected for the study. TPD affected (n=15) and healthy (n=15) trees were identified by tapping observations. Bark samples of 2.5 cm^2 were carefully taken from the scion (tapping panel) and stock of each tree. Latex production was completely absent in the bark tissues from TPD affected scion. The samples were taken to the laboratory on ice and stored at -60°C. Live soft bark tissues were excised and the cytokinins extracted for direct enzyme linked immunosorbant assay (ELISA) after Weiler (1980). The crude extracts prepared by homogenizing 10 g of soft bark in 20 ml of 80 per cent methanol with butylated hydroxytolune (BHT) as antioxident (10 mg/l) were partially purified by passing through polyvinyl polypyrolidone columns. The aqueous extracts were lyophilised and reconstituted with 2 ml of 20 mM tris buffered saline (pH 7.5). The t-ZR contents in the bark tissue samples were analysed by direct ELISA using polyclonal antibodies raised against t-ZR bovine serum albumin (BSA) conjugate (Vonk *et al.*, 1986). The cross reaction of zeatin with the antiserum to t-ZR was found to be 80 per cent with dihydrozeatin riboside and dihydrozeatin about 6 and 4 per cent and less than 1 per cent with isopentnyl adenosin on a molar basis (Shashidhar *et al.*, 1996). Therefore, the cytokinin contents were expressed as t-ZR equivalents (pmol/g fresh weight of the bark tissue). An independent t test was used to compare the healthy and TPD affected trees.