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TAPPING PANEL DRYNESS SYNDROME IN *HEVEA* INCREASES DARK RESPIRATION BUT NOT ATP STATUS

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Tapping panel dryness (TPD) affects the ability of *Hevea* trees to synthesise rubber (cis-poly isoprene) and thus decreases the yield. The present study conducted in *Hevea* clones RRIM 600 and RRII 105 showed that concomitant with an increase in the total sugars and starch contents in the bark, respiration rate also increased but the ATP concentration in the cytosol markedly decreased in TPD affected bark compared to healthy bark from normal trees. This appears to be due to an increase in the non-phosphorylating cyanide resistant alternative respiration in the TPD affected trees.

Key words : *Hevea*, Respiratory pathways, Rubber biosynthesis, TPD.

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INTRODUCTION

Tapping panel dryness (TPD) is generally considered as a physiological disorder commonly noticed in high yielding *Hevea* clones. It is generally believed that when the level of exploitation exceeds the physiological capacity of the tree to generate latex, the tree succumbs to TPD (Chrestin, 1989). The incidence of TPD increases with high tapping frequency and/or excessive yield stimulation (Commere *et al.*, 1989).

Although many studies have been made to describe the development of TPD, the exact cause of the syndrome is not clear. Cytological disorders associated with TPD development were reported by de Fay and Jacob (1981) and Gomez (1990). Studies on viruses and viroids were inconclusive (Peries and Brojier, 1965; Lim, 1973). Dian *et al.* (1995) analysed the changes in the latex protein pattern during the development of this syndrome. Recently Nataraja *et al.* (1998) studied the stress-induced heat stable protein content in the bark tissues of healthy and TPD affected *Hevea* trees. TPD affected bark was observed to have higher levels of sugars, phenols, soluble proteins, peroxidase activity and HMG-CoA reductase activity than normal healthy bark in the *Hevea* clone RRII 105 (Krishnakumar *et al.*, 1999). Chrestin (1985) proposed a biochemical explanation involving laticiferous senescence through activation of oxidative stress leading to dysfunction of the

laticiferous metabolism. The high peroxidase activity and the accumulation of phenols in the TPD affected bark supports this contention (Krishnakumar *et al.*, 1999).

The rubber biosynthesis in TPD affected bark was seriously inhibited although there was adequate supply of carbohydrate substrate for polyisoprene synthesis (Thomas *et al.*, 1998; Krishnakumar *et al.*, 1999). The conversion of mevalonate into polyisoprene is a high energy consuming process (Jacob and Prevot, 1992) and hence energy may be a limiting factor for rubber biosynthesis in the bark tissues of TPD affected trees. It was noticed earlier that there was an increased oxygen consumption in the soft bark tissues of TPD affected plants indicating an increased bark respiratory activity in the TPD affected bark tissues (Krishnakumar *et al.*, 2000). The present study aimed to confirm the respiratory activities in the soft bark tissues using specific respiratory inhibitors and to compare the respiratory activity in the soft bark tissues of normal and TPD affected trees. Along with the measurements of respiratory rates in the soft bark tissue, carbohydrate composition of bark tissues and the concentration of ATP in the latex and bark tissues of TPD affected and healthy trees of two *Hevea* clones (RRII 105 and RRIM 600) were also studied for comparison.

MATERIALS AND METHODS

A 21 year old plantation of RRIM 600 and a 19 year old plantation of RRII 105 from the Central Experiment Station of Rubber Research Institute of India (RRII) at Chethackal (9°22′ N; 76°50′ E) were selected for this study. The trees were under regular tapping, 1/2S d/2 during the two years preceding sampling. Trees with 70 to 90 per cent intensity of TPD and normal trees were identified by tapping observations, during the two months prior to bark sample collection. Bark tissues from the tapping panel of the normal trees (n=10) and from the dry stretches of the tapping panel of TPD affected trees (n=12) were collected separately immediately after tapping. Latex samples (3-5 ml each) were also collected on ice from TPD affected and normal trees, for ATP measurements. Both the bark and latex samples were transported to the laboratory on ice. The required soft bark tissues were carefully removed from these samples and used for measurement of respiration and the remaining bark samples were stored at –60°C for the biochemical analysis.

The tissue oxygen consumption was measured polarographically using a Clark type oxygen electrode (Hanzatech, UK) as directed by Lambers *et al.* (1983) with modifications. Soft bark tissue (approximately 1 mm thick and 200 mg fresh weight) separated from each bark sample was immersed in 2 ml reaction buffer in the electrode chamber. The buffer (pH 7.2) contained 0.3 M sorbitol, 10 mM NaCl, 10 mM KH_2PO_4, 2 mM $MgSO_4$ and 0.1 per cent BSA. The oxygen depletion rate in the assay buffer was recorded for 5 min at 25°C. The tissues were then dried at 70°C and dry weight determined. The rate of oxygen consumption was calculated on a dry weight basis (nM O_2 /min /g dry weight).

To ascertain the rate of cytochrome and alternative oxidase mediated respiration the oxygen consumption of the soft bark tissue was determined after incubating the bark tissues either 300µM KCN or 3mM SHAM for 10 min each in order to inhibit the cytochrome respiratory pathway (Lambers

et al., 1983) or alternative respiratory pathway (Millar *et al.*, 1995) respectively. The tissues were incubated in both these inhibitors one after the other for determining the residual respiratory activity (Millar *et al.*, 1995). The analyses were repeated three times for each tissue sample. Soft bark tissues, 100 mg (fresh weight) was powdered in liquid nitrogen, extracted with 80 per cent ethanol and used for the estimation of total sugars (Scott and Melvin, 1953), and starch (Mc Cready *et al.*, 1950).

The C-serum from the latex samples was prepared by centrifuging the latex at 23,000 rpm for 30 min at 4 ^{0}C. The ATP content in the C-serum of the latex and soft bark tissues of both normal and TPD affected trees were assayed after the method of Fader and Koller (1984) using a luminometer (Stratec Electronic GmbH, Brikenfeld, Germany) and the ATP-bioluminescent kit (FL-AA 89H9803; Sigma Chemical Company, USA).

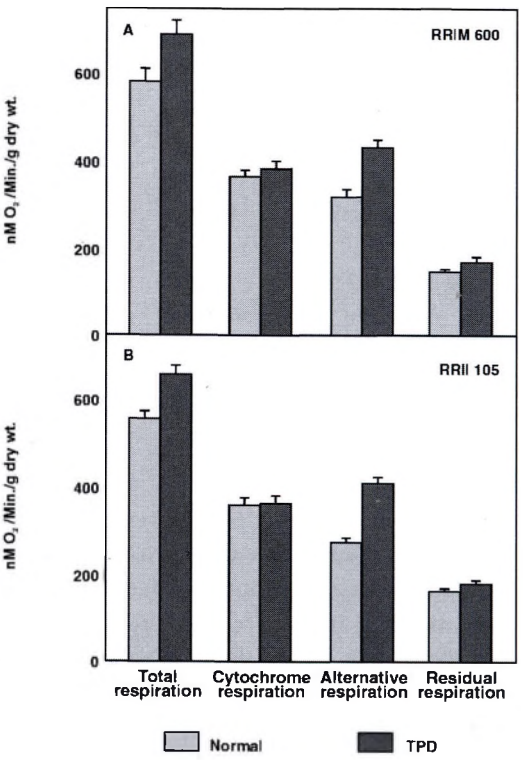

Fig. 1. Respiration in the soft bark tissues of normal and TPD affected *Hevea* trees

RESULTS AND DISCUSSION

The changes in the rate of oxygen consumption in the soft bark tissue after incubating with specific respiratory inhibitors indicate the active tissue respiration in the *Hevea* soft bark (Fig. 1). The cyanide resistant alternative respiratory pathway was significantly higher in the TPD affected bark than in the healthy bark tissue. However, the cytochrome pathway in the bark was not altered due to TPD incidence. The total respiratory rate was more due to the increased alternative respiration in the TPD affected than the healthy bark in both the clones (Fig. 1). This observation was somewhat unexpected due to the previous contention that there would be probably less respiratory activity in a TPD affected bark. It has been shown that supply of carbohydrate was not a limiting factor for rubber biosynthesis (Thomas *et al.*, 1998) but inadequate supply of ATP is possibly the reason for the non-conversion of carbohydrate into isoprene (Krishnakumar *et al.*, 1999). Earlier studies have shown that the TPD affected tissues were experiencing oxidative stress leading to increased peroxidative damage (Krishnakumar *et al.*, 1999). The respiratory rates could go up under stress conditions as a result of enhanced alternative respiratory pathway without concomitant increase in the ATP status of the tissue (Wen and Liang, 1993). TPD affected tissue seems to be physiologically similar to a senescing tissue, which are known to have high respiratory rates as a result of increased alternative

pathway (Wen and Liang 1993). In general, the respiratory rate will increase due to more alternative pathway mediated non-phosphorylating electron transport and the tissues with increased alternative pathway show decreased ATP yield in the cytosol (Millenaar *et al.*, 1998). A general characteristic of alternative respiratory pathway is to produce one molecule of ATP by NAD(P)H oxidation, while three ATP molecules are produced by cyanide sensitive pathway (Lambers *et al.*, 1993).

The total soluble sugar concentration and starch content in the bark tissues were relatively more in TPD affected trees than in healthy trees (Fig. 2). The carbohydrate content and the respiration rate of latex yielding and dry stretches within the tapping panel of TPD affected trees did not show any significant variation (Krishnakumar *et al.*, 2000). Previous studies have shown that

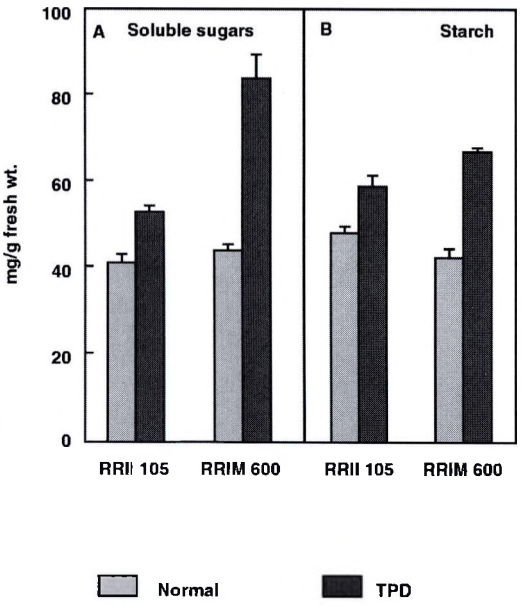

Fig. 2. Total soluble sugar and starch contents in the soft bark tissue of normal and TPD affected *Hevea* trees

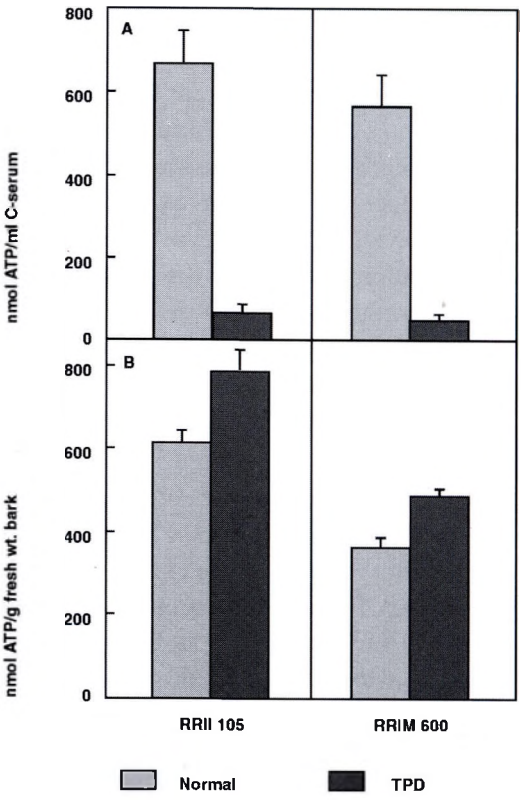

Fig. 3. ATP content in the C-serum and soft bark tissue of normal and TPD affected *Hevea* trees

there was a significant positive correlation between the oxygen consumption rate and total sugar content in the soft bark tissues of *Hevea* (Krishnakumar *et al.*, 2000). Azcon-Bieto *et al.* (1983) suggested a relationship between the rate of respiratory O_2 uptake, the involvement of alternative pathway and the level of carbohydrates in the leaves of wheat. The increased respiratory activity per unit sugar content in the healthy trees is obviously related to the rubber biosynthesis capacity of the bark. The TPD affected trees showed both increased carbohydrate content and respiration in their bark tissue, but no rubber biosynthesis.

It is likely that the altered activity of enzymes such as polyphenol oxidase and peroxidase (Krishnakumar *et al.*, 1999) created some artifacts as residual respiration in the polarographic measurements using the O_2 electrode, but the extent of such interaction will not explain the large increase in respiration observed in the TPD affected bark. Though the residual respiration was remarkably high in *Hevea* bark tissues, there was no significant difference between the healthy and TPD affected bark residual respiration rates (Fig. 1).

There was a remarkable reduction in the ATP status of the C-serum of TPD affected trees but not when measured in the soft bark tissue (Fig. 3). The reduction in the ATP content in the C-serum in spite of an increase in respiration suggests that there was possibly more alternative respiratory pathway (cyanide resistant respiration) operating in the TPD affected bark. The metabolic conversion of mevalonate into isoprene units requires an abundant supply of ATP (Jacob and Prevot, 1992). From the present study it can be presumed that the cytosolic ATP concentration was extremely low in the TPD affected trees which would have adversely affected the capacity of the laticiferous tissue to produce isoprene from carbohydrate, thus causing accumulation of sugars in the tree bark.

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