Research Article

Journal of Plantation Crops, 2014, 42(1): 86-93

Plant hormones and oxidative stress in *Hevea brasiliensis*

R. Krishnakumar*, P.K. Ambily and James Jacob

Rubber Research Institute of India, Kottayam - 686 009, Kerala, India

(Manuscript Received: 12-12-13, Revised: 05-03-14, Accepted: 20-03-14)

Abstract

Plant hormones are naturally occurring organic substances that are produced within the plant at low concentrations which regulate the growth and metabolism. It was observed that over-harvesting latex through high intensity tapping had a direct effect on the endogenous hormone levels in rubber plants. This could induce the development of oxidative stress leading to several complex physiological disorders including tapping panel dryness (TPD). During oxidative stress, the levels of stress hormones increased and the growth hormones decreased in the bark tissue. Both ethylene (ET) and abscisic acid (ABA) concentrations were high in trees that are exposed to oxidative stress. The levels of hydrogen peroxide (H_2O_2) and its scavenging enzyme, peroxidase (Px), present in healthy trees were appeared to be capable of scavenging the H_2O_2 molecule produced in the tissue. Hence, the minimum stress response was noticed in the bark tissues of normal trees. The regular wounding of the bark tissues for harvesting latex cannot be avoided in rubber trees. But, the amount of Px produced in the bark tissue was inadequate to detoxify the H_2O_2 produced under certain physiological state of the tree (TPD) and thus leading to oxidative stress. Accumulation of malondealdehyde (MDA) was evidenced as the peroxidative damage occurred in the bark tissues of stressed trees. The tissue cyanide (CN) level was very high in stressed trees due to the low levels of CN scavenging enzyme, β-cyanolalanine synthase (β-CAS). Trees under oxidative stress had increased levels of stress hormone in the bark tissue and hence, the low levels of growth hormones and high levels of stress hormones in the soft bark tissue would have caused disorders in the cellular differentiation and metabolism in the laticiferous tissues of *Hevea* trees limiting the production leading to significant crop loss.

Keywords: Cyanide, *Hevea brasiliensis,* MDA, oxidative stress, plant hormones, scavenging enzymes

Introduction

Plant hormones at very low concentrations can trigger various physiological and metabolic activities related to growth, differentiation and development. Each group of hormone is known to influence a wide variety of developmental events in plants and most of these events are influenced by more than one group of hormones *viz*., auxins, gibberellins, cytokinins, abscisic acid and ethylene. Ethylene being the hormone regulating the levels of many other hormones and ethylene-mediated signaling events might be triggering the latex cell metabolism and degeneration in *Hevea* (Chen *et al.,* 2002). Tapping-induced ethylene, as well as external ethylene stimulation can influence the levels of other hormones that induce the differentiation of cambium into lacticifers in rubber trees (Chrestin, 1989). The differentiation and regeneration of latex vessels from vascular cambium are under the regulation of hormones such as auxins and cytokinins (Thomas *et al.,* 1995) which are highly coordinated processes bearing latex yield in rubber trees.

There are evidences to suggest that ethylene is the major hormone associated with oxidative stress in plants (Mehlhorn, 1990). Stress hormones can play a major role in the generation of reactive oxygen species (ROS) in biological systems. In plants ROS molecules are produced during photosynthesis and respiration (Maxwell *et al.,* 1999). They are also produced in response to many hormones such as auxin, abscisic acid and salicylic acid. These toxic molecules can cause peroxidative degradation of the cellular membranes, destabilization and lyses of the lutoid membrane and causing oxidative stress in the laticiferous

**Corresponding Author: kkumar@rubberboard.org.in*

tissues of *Hevea* (Jacob and Krishnakumar, 2006). Exogenous application of ethylene compounds and wounding due to tapping can increase the production of endogenous ethylene in normal *Hevea* trees (Krishnakumar *et al.,* 2008). Over-harvesting latex in *Hevea* through ethylene stimulation or high frequency tapping can cause hormonal imbalance that might trigger programmed cell death (PCD) in laticiferus tissues (Nataraja *et al*., 2006).

The scavenging enzymes may detoxify the ROS molecules formed in the latex and lateciferous tissues (Halliwell, 1996). The presences of several scavenging enzymes have already been noticed in *Hevea* tissues (Chrestin, 1989). Hence, a proper coordination of these enzymes are essential for maintaining the physiological health of the tissue, failing which the system may become susceptible to severe oxidative stress and that may lead to various metabolic disorders in *Hevea*. This study was aimed to understand the endogenous levels of hormones such as auxin, gibberellin, abscisic acid, cytokinin and ethylene in the soft bark tissue of mature *Hevea* trees and their crosstalk during the development of oxidative stress.

Materials and methods

The study was carried out in mature trees of *Hevea brasiliensis* (clone RRII 105) grown in the plantation at Rubber Research Institute of India, Kottayam. The trees were under S/2 d3 6d/7 system of tapping for harvesting latex for a period of 11 years. Those trees showing tapping panel dryness (TPD) were considered as trees baring with oxidative stress (Jacob and Krishnakumar, 2006). The rubber trees were monitored during tapping and categorized into two groups as healthy and stress exposed. During tapping, trees producing latex in the tapping panel were considered as normal healthy trees (n=20) and those showing more than 60 per cent dryness in the tapping panel (TPD) as stress exposed trees (n=20).

Bark sampling

Bark samples of two centimeter square size were collected from three different positions of the tree such as root stock (RS), tapping panel (TP) and one feet above the tapping panel (SC) from both stressed and healthy trees. Samples collected from the field were brought to the laboratory in liquid nitrogen and stored at -80 ^{o}C until use. The inner soft bark tissues from each bark sample collected were used for biochemical analyses (Krishnakumar *et al.*, 1999).

Hormonal and biochemical analysis

Wound induced ethylene (ET) produced in the *Hevea* bark tissue was quantified through gas chromatography (Krishnakumar *et al*., 2006). Analysis of auxin (IAA), gibberellin (GA_3), abscisic acid (ABA) and cytokinin (Zeatin) were determined according to Atici *et al.,* 2005. Hydrogen peroxide (H_2O_2) content in the soft bark tissue was determined using commercially available Amplex Red Hydrogen peroxide/peroxidase assay kit from Molecular Probes, The Netherlands. The assay was carried out after the protocol in the assay kit manual (Molecular probes, 2002). The cyanide (CN) content in the bark tissues was determined by resorcinol picric acid method (Drochioin *et al.*, 2003). Estimation of malondealdehyde (MDA) was made using the method of Heath and Packer (1968). The peroxidase enzyme activity in the tissue was determined after the method of Guilbault (1976). The β-Cyanolalanine synthase (β-CAS) enzyme was quantified using the method after Hendrickson *et al*. (1969) and Urbanska *et al*. (2002). Soluble proteins were extracted through cold acetone precipitation of the enzyme extracts and quantified through Bradford (1976) method to determine the specific activities of the enzymes. The components analyzed were compared between experimental and control using statistical tools.

Results and discussion

Plant hormonal analysis

The plant hormones such as ethylene (ET), abscisic acid (ABA), indol acetic acid (IAA), gibberlic acid (GA_3) and zeatin (tZ) in the bark tissues collected from root stock, at tapping panel and in above the tapping panel regions of healthy and stress exposed trees were analysed. Stress indicators like hydrogen peroxide (H_2O_2), peroxidase (Px), malondealdehyde (MDA) cyanide (CN) and β-Cyanolalanine synthase (β-CAS) were also studied in the bark tissues collected from different regions of the tree. In the bark tissue from three different regions of healthy trees showed variations in the ET levels within the tree (Fig. 1).

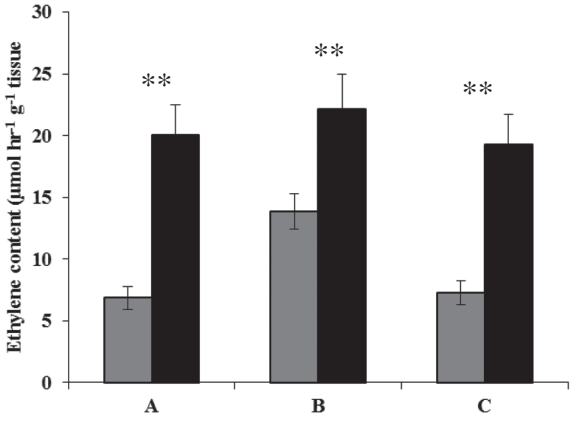

Fig. 1. Ethylene content in different region of healthy (■) and stress exposed (■) *Hevea brasiliensis* A) Root stock; B) Tapping panel; C) Above tapping panel [Values ± SE, ** P ≤ 0.001]

In both groups of trees, ET levels were high in bark tissues collected from the tapping panel compared to the other two regions. In trees which are under oxidative stress, the ET was significantly high in all the three regions compared to healthy trees. However, the ET content in the tapping panel of stressed trees was significantly high compared to other two regions (Fig. 1). The high ET noticed in the tapping panel region of normal trees may be due to wounding of the bark tissue due to regular tapping. The process of wounding is known to stimulate the biosynthesis of ET (Kende, 1993). Compared to healthy trees, wound induced ET was more in trees under oxidative stress. In general, ET is known to induce oxidative stress in plants (Mehlhorn, 1990). The stress exposure of the bark tissue was evidenced through the enhanced tissue respiration and peroxidative damage in rubber trees (Krishnakumar *et al.,* 2000). Since ET regulates the levels of other plant hormones, the overproduction of ET would be associated with several physiological and metabolic changes in the bark tissue of rubber trees.

Comparatively low level of ABA was noticed in healthy trees and the level of ABA was very high in trees exposed to stress. However, ABA was significantly high in the tapping panel region of trees

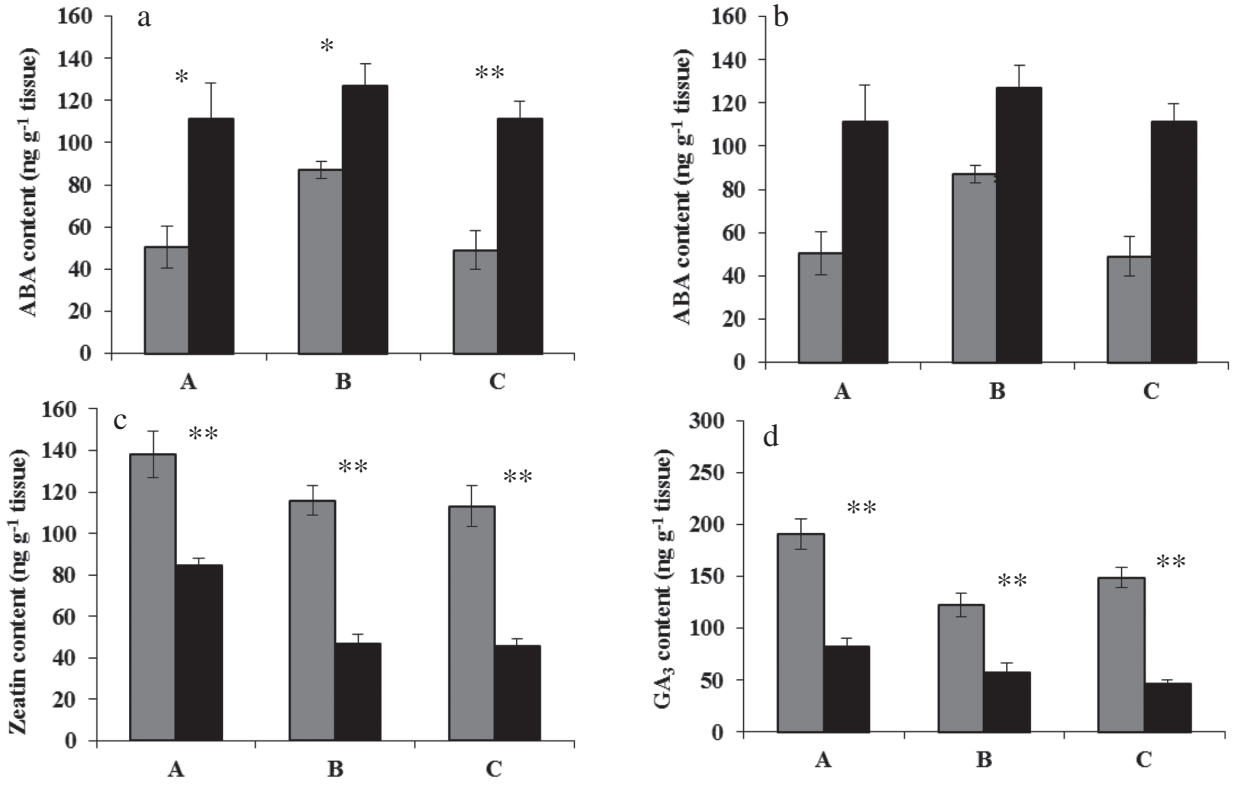

Fig. 2. a) ABA, b) IAA, c) Zeatin, d) GA_3 contents in different regions of healthy (■) and stress exposed (■) *Hevea brasiliensis*. A) Root stock; B) Tapping panel; C) Above tapping panel [Values ± SE, * P≤ 0.05; ** P≤ 0.001; ns= not significant]

of both groups (Fig. 2a). ABA inhibits cell division in the vascular cambium unlike auxin, cytokinin and gibberellins do (Dietz and Hartung, 1998). ET can induce the inter conversion of bound form of ABA to free form as a synergistic effect which may induce ABA mediated metabolism in the tissue.

Root stock tissues had high levels of auxins (IAA), zeatin (tZ) and gibberellin (GA_3) in *Hevea* compared to the two other regions (Figs. 2a to 2d). IAA content was low in the scion of trees exposed to oxidative stress (Fig. 2b). Auxins are the major growth hormone in plants stimulating cell enlargement, differentiation and high meristematic activity. Hence, IAA levels are noticed more in the tapping panel of healthy trees. The low content of IAA present in trees with TPD incidence may be related to the impaired meristematic activity. It has already been reported that in TPD affected trees translocation of phloem sap is regulated by deposition of definitive callose and less differentiation of sieve elements from vascular cambium (Pramod *et al.*, 2011). Similarly, structural modification of phloic rays was noticed in the bark tissues of trees with TPD incidence (Thomas *et al*., 2013). Early observations showed that auxin can reduce the ability of ET to accelerate ageing dependent processes (Lin *et al.,* 2009). Low IAA and high ET levels may lead to impaired lacticiferous differentiation and enhanced senescence in bark tissues, when the trees are exposed to oxidative stress. Cytokinins are generally produced in roots and transported to other parts of the plant. It is interesting to note that a comparatively low level of Zeatin (tZ) in the root stock and the lowest level in the tapping panel region of TPD affected trees (Fig. 2c). However, the healthy trees showed significantly high levels tZ in all the three regions. It has been reported that trans-Zeatin riboside (t-ZR) content in the bark tissue was significantly less in the tapping panel region of TPD affected trees than normal trees (Krishnakumar *et al.,* 1997). Maintenance of high content of t-ZR is therefore essential for the development of pholic elements and active metabolism in the bark tissues including rubber biosynthesis in *Hevea*. In general, the level of GA_3 was low in the tapping panel tissues where wounding becoming a prerequisite for harvesting latex. The occurrence of higher ET and ABA levels in the tapping panel may suppress GA_3 activity. The

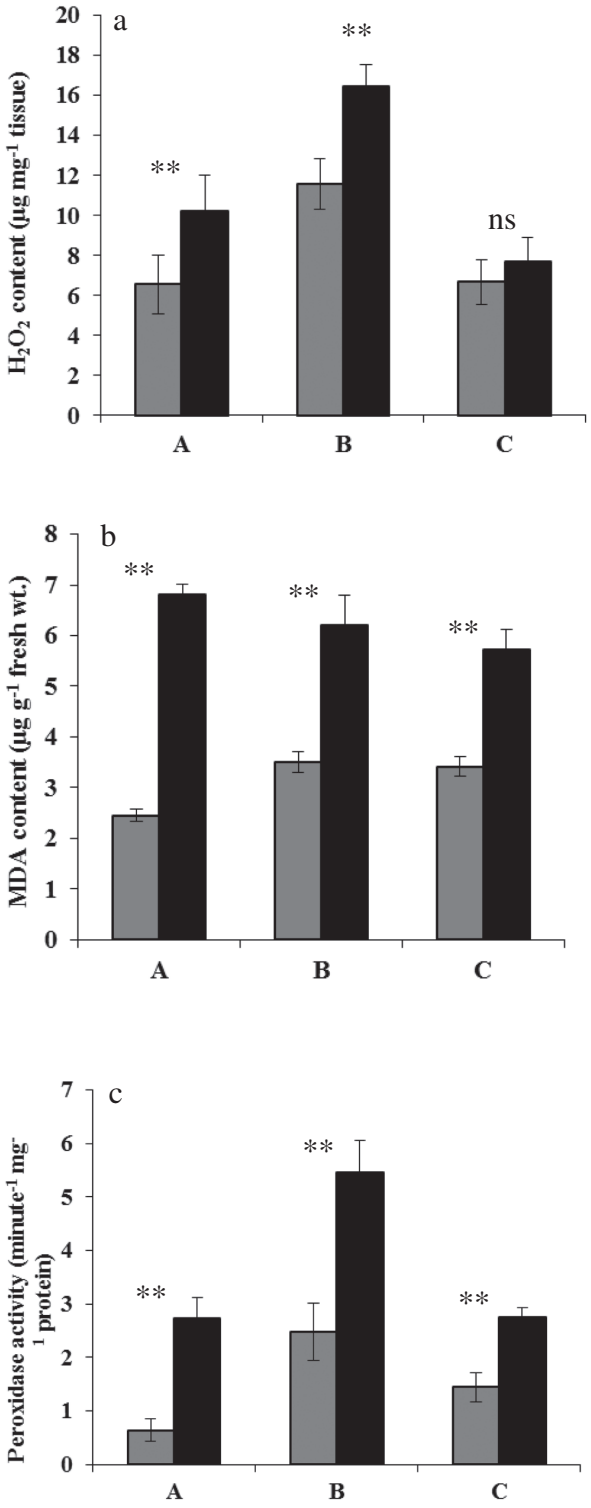

Fig. 3. a) H_2O_2 content, b) MDA content, c) Peroxidase activity in the bark tissues of healthy (■) and stress exposed (■) *Hevea brasiliensis* A) Root stock; B) Tapping panel; C) Above tapping panel [Values ± SE, ns = not significant; ** P ≤ 0.001]

difference in the levels of GA_3 found between healthy and TPD trees (Fig. 2d) indicating low meristematic activity.

Biochemical analysis

Accumulation of hydrogen peroxide (H_2O_2) in the tapping panel of both normal and stress exposed *Hevea* trees indicated the generation of reactive oxygen species (ROS) as a result of wounding through tapping. However, the trees under stress showed high H_2O_2 levels in the rootstock and tapping panel (Fig. 3a). Earlier studies had shown that TPD affected trees experienced oxidative stress as evidenced from peroxidative damage in the laticiferous tissues and enhanced tissue respiration, especially alternate respiratory pathway (Krishnakumar *et al.,* 2001).

Very high MDA content noticed in the bark tissues under oxidative stress (Fig. 3b). The increased MDA levels indicated the peroxidative damage in the tissue due to the ROS action. Hence, MDA accumulation can be attributed to stress induced peroxidative damage of the tissue or tissue organelles. Loss of cell membrane integrity due to oxidative damage was evidenced by the accumulation of MDA in plants (Bartoli *et al*., 1995).

In plants hydrogen peroxide (H_2O_2) is the major ROS produced and it is relatively a stable molecule (Laloi *et al*., 2007). In higher plants H_2O_2 has been recognized as very effective inducer of the transcriptional activity of genes encoding the enzymes of the ET biosynthesis pathway (Jakubowicz *et al.,* 2010). ABA increases the progression of tissue damage and promote H_2O_2 generation which are involved in the induction of peroxidative damage leading to MDA production (Grossmann *et al*., 2001). Therefore, high content of H_2O_2 and ABA in the bark tissues of *Hevea* are detrimental. A cross-talk between the ROS molecules and hormones might provide a suitable condition to stabilize plants under different stress situation.

The total peroxidase activity is very high in the bark tissues collected from the tapping panel, irrespective of the physiological state of the tree. However, significantly high peroxidase activity was noticed in the tapping panel region of stressed trees and the peroxidase activity was minimum in the root stock tissues of normal trees (Fig. 3c). Peroxidase enzyme appears to be capable of scavenging the H_2O_2 produced in the tissues and protecting the cellular organelles from oxidative damage. But, peroxidase activity in the bark tissues of TPD affected trees seems to be inadequate to detoxify the H_2O_2 generated due to the action of stress hormones.

Peroxidase may be a catalytic molecule enhancing ET biosynthesis and IAA degradation in

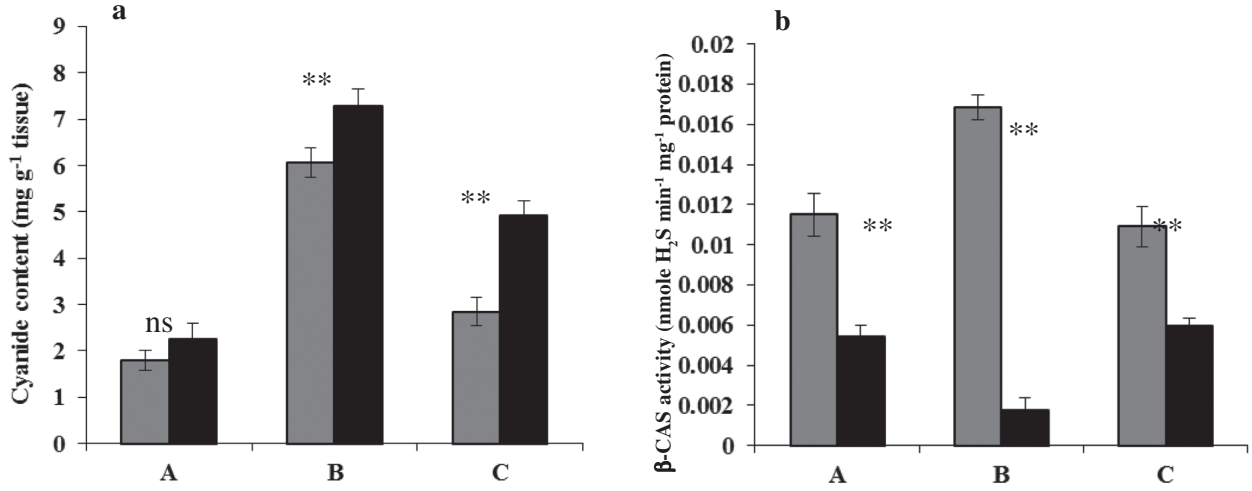

Fig. 4. a) Cyanide content and b) β-CAS activity in the bark tissues of healthy (■) and stress exposed (■) *Hevea brasiliensis*. A) Root stock; B) Tapping panel; C) Above tapping panel [Values ± SE, ns=not significant; **P ≤ 0.001]

the stress exposed *Hevea* trees. It is reported that peroxidase functions as catalyst in the degradation of IAA (Siegel, 1993) and enhances ET biosynthesis (Jakubowicz *et al.,* 2010) in plants. Earlier studies showed a negative correlation of Px activity and t-ZR content in the TPD affected trees (Krishnakumar *et al.,* 2000). Lower levels of cytokinin, higher levels of ET and abnormal rise in the production of ROS in the tissues may lead to the inhibition of mitochondrial activity and hence limiting metabolic activity of the lacticiferous tissues in *Hevea.*

The analysis of cyanide content in the bark tissues from different regions showed significant differences in both normal and stressed trees (Fig. 4a). In both cases the maximum cyanide content was observed in the tapping panel region. The region above the tapping panel showed low levels of cyanide content and the lowest level was noticed in the root stock. High cyanide content was reported earlier in the bark tissues of TPD affected trees (Krishnakumar *et al*., 2006). Prolonged stress in the tapping panel especially over harvesting latex through stimulation, may lead to accumulation of aminocyclopropanecarboxylate oxidase (ACCO) dependent cyanide in the latex producing trees (Chrestin *et al*., 2004). Earlier studies also indicated the possible relation between TPD and cyanogenesis in *Hevea* (Kongswadworakul *et al*., 2006). The decreased IAA and increased ET levels in trees exposed to stress had cross talks between the two hormones leading to the accumulation of CN.

The enzyme β–cyanoalanine synthase (β-CAS), catalyze the formation of β–cyanoalanine from L-cysteine and cyanide, widely seen in plants to detoxify the cyanide accumulation in the tissue. The cyanide detoxifying enzyme was found more in the bark tissues of healthy trees where the cyanide accumulation was minimum in the tissue. On the contrary, the stressed trees had a low level of β-CAS activity in the bark tissues (Fig. 4b). *Hevea* being a cyanogenic plant, the break down of cyanide through β-CAS has a strategic importance. Since the trees were exposed to frequent wounding, formation of endogenous ET and CN in the bark tissue were noticed in high concentrations in the tapping panel region. However, an impaired CN metabolism in the bark tissues was evidenced from the accumulation of cyanide and low β-CAS activity in trees under oxidative stress.

ABA inhibits cell division while both cytokinin and IAA induce cell division and tissue regeneration in plants. Therefore, both groups of plant hormones have antagonistic effects in the regeneration and development of tissues in the tapping panel of *Hevea* trees. However, the high ET-ABA and low tZ-IAA levels noticed in the bark tissues of trees experiencing oxidative stress seem to be alarming and deleterious. Prolonged exposure of this hormonal imbalance in rubber trees may derail metabolic activities leading to oxidative damages. Stress induced ABA production was reported as a major triggering factor for the development of senescence and over production of endogenous ET reported to induce PCD in plants (Nataraja *et al*., 2006). In *Hevea,* changes have been noticed in the five major plant hormones such as ET, ABA, IAA, GA_3 and tZ in the bark tissues along with the production of ROS molecule and scavenging enzymes, under different physiological state of the tree. High intensity tapping and over harvesting latex may contribute the development of oxidative stress in *Hevea*. Hence, plant hormones may play a major role in maintaining the tree health or impaired the tissue metabolism. This study indicated that several factors are influencing the cross-talk between the major plant hormones and their regulation in the metabolic activity of the tree.

Acknowledgment

The authors gratefully acknowledge Ms. Sujisha T.C for her help provided in the hormone analysis and Dr. K. Annamalainathan, Dy. Director (Crop Physiology) for his valuable suggestions extended to this investigation.

References

Atici, O., Agar, G. and Battal, P. 2005. Changes in phytohormone contents in chickpea seeds germinating under lead or zinc stress. *Biologia Plantarum* **49**(2): 215-222.

Bartoli, C.G., Simontacchi, M., Cuiamet, J.J., Montaldi, E. and Puntarulo, S. 1995. Antioxidant enzymes and lipid-peroxidation during aging of *Charusanthemum-morifolium* ram petals. *Plant Science* **104**(2): 161-168.

Bradford, M.M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing

the principle of protein dye binding. *Analytical Biochemistry* **72**: 248-254.

Chrestin, H. 1989. Biochemical aspects of bark dryness induced by over-stimulation of rubber trees with ethrel. In: *Physiology of Rubber Tree Latex* (Eds. J.d'Auzac, J.I., Jacob and Chrestin), CRC Press, Florida, USA, pp. 431-442.

Chrestin, H., Sookmark, U., Trouslot, P., Pellegrin, F. and Nandris, D. 2004. Rubber tree (*Hevea brasiliensis*) bark necrosis syndrome 3: A physiological disease linked to impaired cyanide metabolism. *Plant Disease* **88**(9): 1047.

Chen, S., Peng, S., Huang, G., Wu, K., Fu, X. and Chen, Z. 2002. Association of decreased expression of a Myb transcription factor with the TPD (tapping panel dryness) syndrome in *Hevea brasiliensis*. *Plant Molecular Biology* **51**(1): 51- 58.

Dietz, S. and Hartung, W. 1998. ABA in lichens: Variation, water relations and metabolism, *New Phytologist* 138: 99-106.

Drochioin, G., Oniscu, C., Sanely, V., Popa, K., Cuciac, C and Cozma, D. 2003. Cyanide assay based on its novel reaction with resorcinol and picric acid. *The European Journal of Mineral Processing and Environmental Protection* **3**(3): 291-296.

Grossmann, K., Kwiatkoswki, J. and Tresch, S. 2001. Auxin herbicides induce H_2O_2 overproduction and tissue damage in cleavers (*Galium aparine* L.). *Journal of Experimental Botany* **52**: 1811-1816.

Guilbault, G.G. 1976. Handbook of Enzymatic Methods of Analysis. Marcel dekker, Inc., New York. pp. 147.

Halliwell, B. 1996. Cellular stress protection metabolism. *Biochemical Society Transactions* **24**: 1023-1027.

Heath, R.L. and Packer, L. 1968. Photoperoxidation in isolated chloroplasts. 1. Kinetics and stoichiometry of fatty acid peroxidation. *Archives in Biochemistry and Biophysics* **125**: 175-180.

Hendrickson, H.R. and Conn, E.E. 1969. Cyanide metabolism in higher plants IV. Purification and properties of the â-cyanoalanine synthese of blue lupine. *Journal of Chemistry* **224**: 2632-2640.

Jakubowicz, M., Galgañska, H., Nowak, W. and Sadowski, J. 2010. Exogenously induced expression of ethylene biosynthesis, ethylene perception, phospholipase D, and Rboh-oxidase genes in broccoli seedlings. *Journal of Experimental Botany* **61**(12): 3475-91.

Jacob, J. and Krishnakumar, R. 2006. Tapping panel dryness syndrome: What we know and what we do not know. In: *Tapping Panel Dryness of Rubber Trees* (Eds.) James Jacob, R. Krishnakumar and N.M. Mathew, Rubber Research Institute of India, Kottayam, India, pp. 3-27.

Kende, H. 1993. Ethylene biosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* **44**: 283-307.

Kongswadworakul, P., Sookmark, U., Nandris, D. and Chrestin, H. 2006. Cyanide metabolism and molecular approach to studies on trunkphloem necrosis. In: *Tapping Panel Dryness of Rubber Trees* (Eds.) James Jacob, R. Krishnakumar and N.M. Mathew, Rubber Research Institute of India, Kottayam, India, pp.93-105.

Krishnakumar, R., Ambily, P.K., Raghavan R., Annamalainathan, K. and Jacob, J. 2008. Ethylene stimulation aggravates tapping panel dryness in partially affected *Hevea* trees. *Journal of Plantation Crops* **36**(3): 315-321.

Krishnakumar, R., Annamalinathan, K., Simon, S.P. and Jacob, J. 2000. TPD syndrome increases bark respiration in *Hevea*. In: *Recent Advances in Plantation Crop Research* (Eds.) N. Muraleedharan and R. Raj Kumar, Allied Publishers Limited, New Delhi, pp. 241-245.

Krishnakumar, R., Cornish, K. and Jacob, J. 2001. Rubber biosynthesis in tapping panel dryness affected *Hevea* trees. *Journal of Natural Rubber Research* **4**(4): 131-139.

Krishnakumar, R., Mathew, R., Sreelatha, S. and Jacob, J. 2006. Endogenous ethylene and oxidative stress in *Hevea brasiliensis*. In: *Tapping Panel Dryness of Rubber Trees* (Eds.) James Jacob, R. Krishnakumar and N.M. Mathew, Rubber Research Institute of India, Kottayam, India, pp. 116-124.

Krishnakumar, R., Sasidhar, V.R. and Sethuraj, M.R. 1997. Tapping panel dryness lowers the cytokinin (t-ZR) content in *Hevea* bark tissue. *Indian Journal of Natural Rubber Research* **10**(1&2): 107-109.

Laloi, C., Stachowiak, M., Pers-Kamczyc, E., Warzych, E., Murgia, I., Apel, K. 2007. Cross-talk between singlet oxygen- and hydrogen peroxide-dependent signaling of stress responses in *Arabidopsis thaliana*. *Proceedings of National Academy Science* **104**: 672–677.

Maxwell, D.P., Wang, Y. and McIntosh, L. 1999. The alternative oxidase lowers mitochondrial reactive oxygen production in plant cells. *Proceedings of National Academy of Science* **96**: 8271-8276.

Mehlhorn, H. 1990. Ethylene- promoted ascorbate peroxidase activity protects plants against hydrogen peroxidase activity protects plants against hydrogen peroxide, ozone and parawuant. *Plant, Cell and Environment* **13**: 971-976.

Nataraja, K.N., Prasad, T.G., Udayakumar, M., Sathik, M.B.M.,

Thomas, M. and Jacob, J. 2006. Hormone conflit: Can it be responsible for post differentiation degeneration of latex vessels leading to tapping panel dryness in *Hevea brasiliensis*?. In: *Tapping Panel Dryness of Rubber Trees* (Eds.) James Jacob, R. Krishnakumar and N.M. Mathew, Rubber Research Institute of India, Kottayam, India, pp. 125-129.

Pramod, S., Thomas, V., Rao, K.S. and Krishnakumar, R. 2011. Definitive callose deposition in tapping panel dryness affected bark of *Hevea brasiliensis. Journal of Sustainable Forestry* **30**: 329-342.

Siegel, B.Z. 1993. Plant peroxidases – An organismic perspective. *Plant Growth Regulation* **12**: 303-312.

Thomas, V., Premakumari, D., Reghu, C.P., Panikar, A.O.N. and Saraswathyamma, C.K. 1995. Anatomical and histochemical aspects of bark regeneration in *Hevea brasiliensis*. *Annals of Botany* **75**: 421-426.

Thomas, V., S. Pramod and Rao, K.S. 2013. Structural modification of phloic rays in *Hevea Brasiliensis* with reference to tapping panel dryness and stimulation. *Journal of Plantation Crops* **41**: 142-150.

Urbanska, A., Leszczynski, B., Matok, H., Dixson, A.F.G. 2002. Cyanide detoxifying enzymes of bird cherry oat aphid. *Electronic Journal of Polish Agricultural Universities, Biology* **5**: 1-6.

Lin, Z., Zhong, S. and Grierson, D. 2009. Recent advances in ethylene research. *Journal of Experimental Botany* **60**(12): 3311-3336.