Natural Rubber Research, **23** (1&2): 55-63, 2010

DROUGHT-INDUCED CHANGES IN PHOTOSYNTHESIS AND CHLOROPLAST PROTEINS IN YOUNG PLANTS OF *HEVEA BRASILIENSIS*

K. Annamalainathan, Genu George, Susan Joy, Sony Thomas and James Jacob

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

Annamalainathan, K., George, G., Joy, S., Thomas, S. and Jacob, J. (2010). Drought-induced changes in photosynthesis and chloroplast proteins in young plants of *Hevea brasiliensis*. *Natural Rubber Research*, **23**(1&2):55-63.

One-year-old plants of *Hevea brasiliensis* belonging to four clones, viz. RRII 105, RRII 430, RRIM 600 and PB 260, grown in big-sized polybags, were subjected to water deficit stress by withholding irrigation for 18 days during two consecutive summer seasons (March, 2008 and March, 2009). A consistently over-expressing 23 kDa stress protein in the chloroplast was observed in plants stressed by drought and high light intensity. The amino acid sequence of the stress protein was already elucidated and found to be a small chloroplast heat shock protein (sHSP). The magnitude of the expression level of this stress protein was relatively high in drought-tolerant clones indicating the probable role of this protein in abiotic stress tolerance. The stress tolerance traits in these rubber clones were analyzed by measuring various photosynthetic parameters. There was a significant reduction in photosynthetic oxygen evolution rate in the leaves of drought imposed plants. On the contrary, dark respiration of leaf was increased during early drought period. Further, the maximum potential (Fv/Fm) and effective quantum yield of PS II (ΦPS II) and electron transport rate were drastically inhibited in drought-imposed plants. However, the clones RRII 430 and RRIM 600 recorded relatively small inhibition in ΦPS II and photosynthetic rate as compared to other clones which can be attributed to their inherent drought-tolerant characters. The clones RRII 105 and PB 260 were shown to be drought susceptible as determined from their photosynthetic parameters and expression level of sHSP.

Keywords: Drought, Effective quantum yield of PS II, High solar light, HSP, Photosynthesis

INTRODUCTION

Hevea brasiliensis is the most important commercial source of natural rubber (NR). Owing to the increasing global demand for NR and its limited scope for expansion in the traditional belts, attempts are being made to extend the cultivation to marginally suitable areas in most rubber growing countries with varied climatic constraints like moisture stress and high and low temperature. In India, cultivation of rubber is being extended to North East India. Drought, combined with high solar light intensity, has been reported as a major environmental constraint for establishing rubber cultivation in areas such as the North Konkan region of India (Jacob *et al.*, 1999; Alam *et al.*, 2005). Most of the field-grown plants tolerate environmental stresses through many metabolic adaptations at cellular level. In general, most of the damaging effects of irradiation and moisture stress to green leaves occur at the chloroplast membrane and enzyme levels (Oquiset *et al.*,

Correspondence: K. Annamalainathan (Email: annamalai@rubberboard.org.in)

1995). The PS II and electron transport components of thylakoid membranes are the main targets of photoinhibition due to the formation of excess reactive oxygen species during adverse climatic condition (Demmig-Adam and Adams, 1992). Drought and high intensity light drastically inhibit light reactions and damage the thylakoid proteins in young plants of *Hevea* (Annamalainathan *et al.*, 2006). Drought tolerance of crop plants can be considered as the tolerance of moderate level dehydration.

The responses to stress have been documented in many different biological systems. A common feature of this response is the induction of a group of proteins which were first termed as "heat shock proteins" due to their initial discovery in cells exposed to hyperthermia. Generally the induced proteins are known as "stress proteins," ranging in size approximately from 15 to 110 kDa in molecular weight. Some of these proteins are constitutive (found in cells under normal conditions), while others have been found to be expressed under a variety of cellular stresses including heavy metals, high temperature, drought and light-mediated oxidative stress (Vierling, 1991; Heckathorn, *et al.*, 2004). Many stress proteins seem to function as molecular chaperons by regulating protein folding, while others play a role in regulating the function of receptors. In chloroplast, the small chloroplast heat shock proteins (sHSP) have been implicated in protecting this organelle from photoinhibitory and oxidative stress by preventing protein aggregation and stabilizing thylakoid membrane (Torok *et al.*, 2001). Recent study has reported that chloroplast sHSPs also protect photosynthetic electron transport from inhibitory effects of heavy metals (Heckathorn *et al.*, 2004).

The objectives of the present study were to find out the clonal response of young *Hevea* plants to drought and to analyse stress protein expression and its relevance in drought tolerance. The plants were evaluated for drought tolerance by analyzing certain physiological activities like photosynthetic oxygen evolution, dark respiration, maximum and effective quantum yield of PS II activities and electron transport rate (ETR) of PS II. The expression level of sHSP and its possible relationship with drought tolerance mechanism are also analyzed.

MATERIALS AND METHODS

Planting material and growth condition

Budded stumps of four clones of *Hevea*, namely RRII 105, RRIM 600, RRII 430 and PB 260, were planted in large size polythene bags (65x35 cm). The plants were grown under normal field conditions (20 plants per treatment) with open sunlight. One set of plants in each clone was drought-stressed by withholding irrigation for 18 days during the rain-free summer season of 2008 and 2009 and a second set was kept as irrigated control. Photosynthetic studies were conducted after 15 days of withholding irrigation. For chloroplast protein analysis leaf samples were collected on the 18th day of withholding irrigation.

Water potential

The water potential of the leaf was measured before sampling by using Psypro water potential system - Wescor (435 752 6011). Psypro meter measures the water vapor pressure of a solution or plant sample, on the basis of the principle that evaporation of water from a surface cools the surface. The sample chambers of Wescor system were taken to the field and the collected leaf discs

were immediately transferred to the chambers, transported to the laboratory and then observations were taken.

Measurements of photosynthesis and respiration

The rate of photosynthetic oxygen evolution by the freshly harvested leaf discs (with an area of 9.2 cm^2) was measured at 25 ^0C with a Clark type oxygen electrode (Hansatech LD 2/2, King's Lynn, UK). The measurement of light (LED) was achieved using a Hansatech LH 36 light source. To avoid any CO_2 limitation, 2% CO_2 was generated in the closed chamber by a bicarbonate buffer (pH 9.2). The leaf disc was first acclimatized to dark for five minutes and the rate of dark respiratory oxygen uptake was measured. The leaf disc was then exposed to different light intensities (200 and 400 µmol/m^2/s) using an LED source (LH 36, Hansatech, UK) for 5 min each and photosynthetic oxygen evolution rate was measured at 25 ^0C (Walker, 1988).

Assay of quantum yield of PS II

Chlorophyll fluorescence measurements were made following standard technique as proposed by Schreiber *et al.*, (1998). Chlorophyll fluorescence parameters namely, maximum potential rate of photosystem II (dark Fv/Fm), minimal (Fo') and maximal (Fm') fluorescence under light exposure, effective PS II quantum yield (Φ PS II), efficiency of excitation energy capture by open PS II reaction centre after exposure to 316 µmol actinic light and electron transport rate of PS II (ETR) were measured by using PAM 2100 and Dual-PAM 100 (Walz, Germany), (Schreiber *et al.*, 1998).

Isolation of chloroplasts

Type II broken chloroplasts were isolated by the method of Fish and Jagendorf (1982). Fresh leaf sample was ground with liquid nitrogen in mortar and pestle. The powdered leaf sample was added with 5 ml of grinding buffer and transferred to a centrifuge tube. The homogenate was centrifuged at 500 g for 2 min. The pellet represented unbroken cells and tissue was removed and the supernatant was spun at 3500 g for 5 min and the resulting pellet was resuspended in 1 ml of Tris buffer (pH 7.8) as chloroplast suspension.

Protein preparation and analysis in SDS-PAGE

Chloroplasts were precipitated with 10% TCA and kept on ice for 30 min before centrifugation to collect the pellet. Traces of the TCA in the pellet were removed by repeated washing (three times) with ice cold acetone. The final pellet was air-dried and solubilised in a small amount of 10% SDS to which equal volume of sample buffer was added. The samples were boiled for 2 min and centrifuged at 3000 g for 5 min to remove insoluble materials. Chloroplast proteins were dissolved in 10% SDS and quantified by the method of Lowry *et al.* (1951). Analysis of chloroplast protein was carried out by SDS-PAGE according to the method of Laemmli (1970) using a 12% linear gel.

RESULTS AND DISCUSSION

The drought-imposed plants showed typical symptoms such as leaf discoloration and leaf lamina drying. There were clear differences in the degree of drought symptoms among the *Hevea* clones studied. The clone RRII 105 showed stunted growth with heavy chlorophyll bleaching in leaf lamina and drying of the lower leaves. The clone PB 260 was also severely affected by drought as evidenced from morphological

appearance. The clones RRIM 600 and RRII 430 showed a certain degree of drought tolerance as these plants were visibly not much affected, except for minor drying in leaf tips. The morning leaf water potential (Ψ_w) of irrigated plants was in the range of -1.7 to -1.9 MPa whereas the drought-imposed plants recorded significantly lower water potential in the range of -2.3 to -2.6 MPa. However, there was no significant difference among the clones (data not shown).

Further, photosynthetic oxygen evolution, dark respiration and PS II activities were measured in irrigated and drought-imposed plants to ascertain the magnitude of drought effect on photosynthetic apparatus. Except RRII 430, other clones recorded a marginal increase in dark respiration rate after imposition of drought. Probably, this is the general response in young plants to early water deficit stress under high light conditions (Fig. 1). In general, respiration rates have been shown to increase in most plants in the early stage of water deficit. However, in later stage, the respiration rate tends to decrease when drought condition is intensified (Jouve *et al.*, 2007). Under mild water stress condition, the oxidation rate of carbohydrates may increase up to certain levels, following which plants might succumb to severity of stress.

There was significant reduction in photosynthetic oxygen evolution rate in all the four clones under drought condition (Fig. 2). Indeed, significant differences existed in the degree of reduction in oxygen evolution rates among the clones. After 15 days of withholding irrigation, RRII 105 recorded 70% reduction in photosynthetic rate in drought-imposed plants as compared to irrigated plants, followed by PB 260, RRIM 600 and RRII 430. The inhibition of photosynthetic oxygen evolution rate was the least in RRII 430. The popular clone RRII 105 was more susceptible to soil moisture deficit (Annamalainathan *et al.*, 2005; Alam *et al.*, 2005). However, the clones RRII 430 and RRIM 600 were relatively tolerant to drought and high light as observed from a small reduction in photosynthesis (Fig. 2). In many crop plants, photosynthetic adaptation to water deficit and high solar

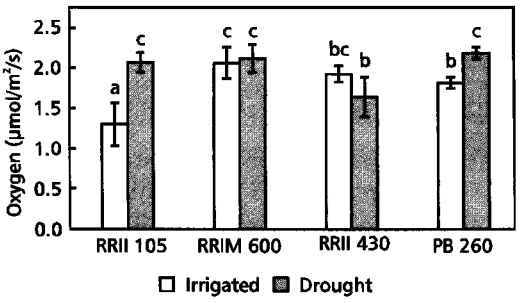

Fig. 1. Dark respiration rate in leaves of young plants of four clones of *Hevea* grown under irrigated and drought conditions. Different alphabets indicate significant difference at 5% level.

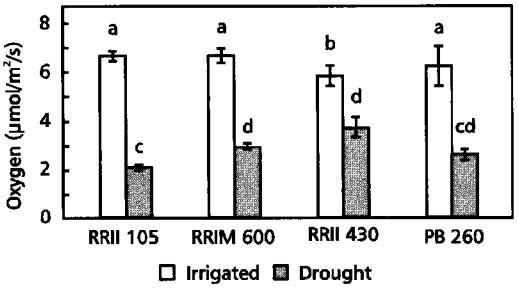

Fig. 2. Photosynthetic oxygen evolution rate in four clones of *Hevea* grown under control and drought conditions. The measurement light was 400 µmol/m²/s red actinic illumination provided from a LED source. Different alphabets indicate significant difference at 5% level.

radiation was explained at the level of chloroplast electron transport components and membrane organization (Anderson and Barber, 1996). Photosynthetic activities are very much sensitive to prevailing environmental conditions and influenced by water deficit and high solar light conditions (Anderson 1986). The reduction in photosynthetic rate was directly related to chlorophyll and Rubisco contents also. The Rubisco content has been found to be reduced under drought and high light conditions in *Hevea* (Annamalainathan *et al.*, 2005).

The maximum potential quantum yield of healthy plants (dark Fv/Fm) was in the range of 0.79 - 0.81. Compared to irrigated control plants, the drought plants recorded a significant reduction in dark Fv/Fm (Fig. 3). The reduction was maximum in RRII

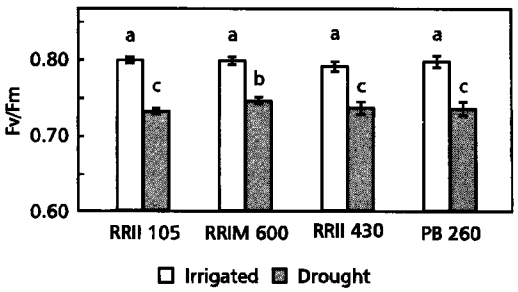

Fig. 3. Maximum potential quantum yield of photosystem II (Dark Fv/Fm) rate in four clones of *Hevea* grown under irrigated and drought conditions. Different alphabets indicate significant difference at 5% level.

105 and PB 260 and minimum in RRIM 600. Similar reduction in Fv/Fm rate has been observed in many clones of *Hevea* in a severe drought-prone area in Maharashtra (Alam *et al.*, 2005). A reduction in dark Fv/Fm in leaves is related to inhibition of PS II activity (Jacob *et al.*, 1999). Any environmental perturbation is quickly reflected in the activity of PS II (Adams *et al.*, 2001). Similarly the effective quantum yield of PS II (Φ PS II) in drought-imposed plants was significantly inhibited. There was a severe inhibition of Φ PS II in clones RRII 105 and PB 260 compared to RRII 430 and RRIM 600 (Fig. 4). The

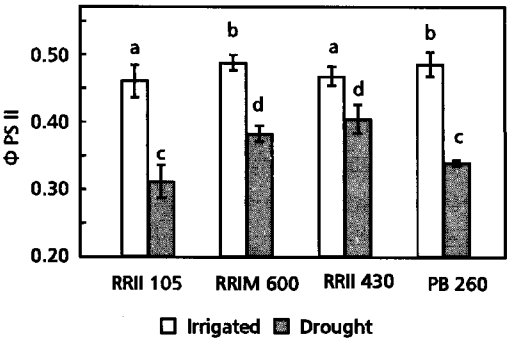

Fig. 4. Effective quantum yield of PS II (ΦPS II) in four clones of *Hevea* grown under irrigated and drought conditions. The red actinic light was (216 μmol/m^2/s) illuminated for four minutes before the measurement of ΦPS II. Different alphabets indicate significant difference at 5% level.

effective quantum yield is a crucial parameter typically indicating the actual efficiency of photosynthesis in illuminated leaf (Asada, 1999; Munne-Bosch and Algre, 2000). Therefore, any reduction in Φ PS II directly affects PS II activities and subsequent carboxylation reactions. The electron transport rate (ETR) of PS II was calculated from Φ PS II and the intensity of actinic light illumination. There was a significant reduction of ETR in drought imposed plants as compared to control plants (Fig. 5). The clone RRII 105 recorded a drastic reduction in ETR that was reflected in photosynthetic

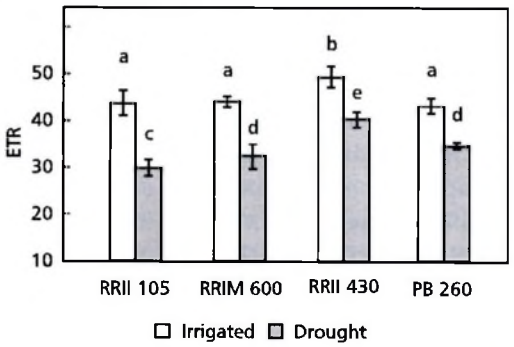

Fig. 5. Electron transport rate (ETR) of PS II in four clones of *Hevea* grown under irrigated and drought conditions. Different alphabets indicate significant difference at 5% level.

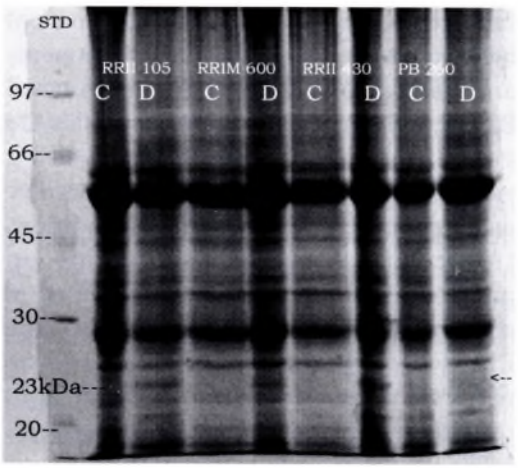

Fig. 6. SDS-PAGE profile of chloroplasts proteins. Proteins were prepared from chloroplasts of irrigated (C) and drought imposed (D) young plants of *Hevea brasiliensis*. The numbers at left side of the figure indicate standard molecular weight markers (kDa) and stress protein (23 kDa).

oxygen evolution rate also (Fig. 2). The clone RRII 430 showed comparatively stable ETR under drought conditions. Efficiency in the utilization of absorbed light energy in the leaf, in general, is manifested in photochemical activities and ETR across PS II (Adams *et al.*, 2001). The combined effect of high light and drought stress on photosynthetic oxygen evolution rate was more pronounced than the individual effects in *Hevea* (Annamalainathan *et al.*, 2005). The reduction in photosynthetic rate occurred in field during unfavorable climatic conditions was proved to be due to the damaging effects of active oxygen species (AOS). The AOS and singlet oxygen species have been reported to inflict damage to the thylakoid membranes. The PS II, thylakoid membranes and electron transport components are the main targets of photoinhibition due to the formation of excess active oxygen species during adverse climatic conditions (Halliwell and Gutteridge, 1999; Demmig-Adams and Adams, 2006).

The chloroplast protein profile (Fig. 6) shows induction of low molecular weight proteins, specifically a 23 kDa protein. There was a marked reduction in large subunit (55 kDa) of Rubisco content in the chloroplast protein profile in RRII 105. The 23 kDa small chloroplast protein was identified in thylakoid membranes of *Hevea* and the amino acid sequence revealed that it was a heat shock protein specific to *Hevea* (Annamalainathan *et al.*, 2006). Among the four clones, RRII 430 recorded a prominent expression of this protein. This particular protein was not observed in PB 260, most probably, due to very low expression level not visible under coomassie blue staining.

In the present study, a consistently over-expressing 23 kDa protein was observed in three clones of young *Hevea* plants experiencing drought concomitant with high solar light intensity. Stress proteins are

synthesized by the cell in response to any environmental influence that has a dehydration component, such as drought, salinity or extra cellular freezing (Ingram and Bartels, 1996). It may stabilize macromolecules through chaperon-like properties and may act synergistically with compatible solutes (Hoekstra *et al.*, 2002). While present in most eukaryotes, the sHSP class appears to be the most prevalent in higher plants alco. The amino acid sequence revealed that the *Hevea* sHSP is a novel protein and not reported in other species as the sequencing homogeneity is only partial with other reported sHSPs (Annamalainathan *et al.*, 2006). In general, the chloroplast sHSPs have been reported in a variety of plant species, including a 26 kDa HSP from tomato, *Arabidopsis* and soybean (Suzuki *et al.*, 1998). Coordinated expression of late embryogenesis abundant proteins (LEA) and sHSPs transcript was observed during embryo development in response to ABA, indicating the existence of common regulatory elements for both LEA proteins and sHSPs (Wehmeyer *et al.*, 1996). Moreover, a great deal of evidence indicates that sHSPs play a role in tolerance to a variety of biotic and abiotic stresses as well as key development processes (Vierling, 1991; Heckathorn *et al.*, 2004). Heckathorn *et al.* (2004) have reported that this protein is involved in the protection of PS II when the plants experience abiotic stresses, including that of metal toxicity.

In chloroplast, the sHSPs have been implicated in protecting chloroplast from photoinhibitory and oxidative stress by preventing aggregation and stabilizing the thylakoid membrane (Torok *et al.*, 2001). It has been demonstrated that the chloroplast sHSPs play a direct role in stabilizing the photosystem (PS II) oxygen evolution complex proteins during heat stress and thereby promote the maintenance of PS II electron transport. The steady state levels of major PS II proteins, including the D1and D2 proteins in the PS II reaction centre, declined with increasing severe water deficit, possibly as a result of increased degradation of stress proteins (Torok *et al.*, 2001). Thus sHSP in chloroplasts of *Hevea* appears to be a general stress protein that may have a role in protecting thylakoid membrane from oxidative stress and in maintaining other functions leading to the survival of this organelle during stress or facilitating recovery from stress.

In the present study, the expression level of sHSP was greater in those clones of *H. brasiliensis* which showed consistently stable photosynthetic rate as well as PS II activities under water deficit condition. These observations clearly indicated that the stress protein might have a role in chloroplast, probably involving in the protection of PS II and other thylakoid membrane electron transport components from the photo oxidative damage. Further, the expression level of this protein may be considered as a biochemical marker in the screening programme for early evaluation of various clones of *H. brasiliensis* for their drought tolerance potential.

ACKNOWLEDGEMENT

The authors thank Dr. R. Krishnakumar, Joint Director, Crop Physiology Division, Rubber Research Institute of India, Kottayam for support and encouragement.

REFERENCES

Annamalainathan, K., Nair, D. B. and Jacob, J. (2005). Effect of light and drought on the photosynthetic apparatus of young rubber plants (*Hevea brasiliensis*). *In: Stress Biology*. (Eds. U. Chakraborty and B. Chakraborty) Narosa Publishing House, New Delhi. pp. 57-62.

Annamalainathan, K., Heazlewood, J. L. and Jacob, J. (2006). Studies on chloroplastic factors responsible for drought tolerance in rubber plants. *Journal of Plantation Crops*, **34**(3): 643-649.

Alam, B., Annamalainathan, K. and Jacob, J. (2005). Light quintessentially modulates the impact of drought stress on photosynthesis in the leaves of field grown *Hevea brasiliensis*. *Proceedings of International Natural Rubber Conference*, India, pp. 239-245.

Anderson, J. M. (1986). Chlorophyll fluorescence yield changes as a tool in plant physiology. *Annual Review of Plant Physiology*, **37**: 93-136.

Anderson, B. and Barber, J. (1996). Mechanisms of photodamage and protein degradation during photoinhibition of photosystem II. In: *Baker NR, edition. Photosynthesis and the Environment.* Kluwer Academic Publishers, Dordrecht, The Netherlands, pp.101-121.

Adams, W. W. III., Demmig-Adams, B., Rosenstile, T. N. and Albert, V. (2001). Dependence of photosynthesis and energy dissipation activity upon growth form and light environment during winter. *Photosynthesis Research*, **76**: 51-62.

Asada, K. (1999). The water-water cycle in chloroplast; Scavenging of active oxygen and dissipation of excess photons. *Annual Review of Plant Physiology and Plant Molecular Biology*, **50**: 601-639.

Demmig-Adams, B. and Adams W. W. III. (1992). Photoprotection and other response of plants to high light stress. *Annual Review of Plant Physiology and Molecular Biology*, **43**: 599-626.

Demmig-Adams, B. and Adams, W. W. III. (2006). Photoprotection in an ecological context. The remarkable complexity of thermal energy dissipation. *New Phytologist*, **172**: 11-21.

Fish, L. E. and Jagendorf, A. T. (1982). High rates of protein synthesis by isolated chloroplast. *Plant Physiology*, **70**: 1107-1114.

Halliwell, B. and Gutteridge, J. M. C. (1999). Free Radicals and Other Reactive Species in Disease, Free Radicals in Biology and Medicine, 1999-doi.wiley.com.

Heckathorn, S. A., Muller, J. K., LaGuidiece, S., Zhu, B., Barret, T., Blair, B. and Dong, Y. (2004). Chloroplast small heat shock proteins protect photosynthesis during heavy metal stress. *American Journal of Botany*, **91**: 1312-1318.

Hoekstra, F. A., Golovina, E. A. and Buitink, J. (2002). Mechanisms of plant desiccation tolerance, *Science*, **6**: 431-438.

Ingram, J. and Bartels, D. (1996). The molecular basis of dehydration tolerance in plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, **47**: 377-403.

Jacob. J, Annamalainathan, K., Alam, B., Sathik, M.B.M., Thapliyal, A. P. and Devakumar, A. S. (1999). Physiological constraints for cultivation of *Hevea brasiliensis* in certain unfavourable agroclimatic regions of India. *Indian Journal of Natural Rubber Research*, **12** (1&2): 1–16.

Jouve, L., Jacques, D., Douglas, G. C., Hoffmann, L. and Hausman, J. (2007). Biochemical characterization of early and late bud flushing in common ash (*Fraxinus excelsior L*). *Plant Science*, **172**: 962-969.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**: 680-685.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R.J. (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry*, **193**: 265-75.

Munne-Bosch, S. and Alegre, L. (2000). Changes in carotenoids, tocophenols and diterpens during drought and recovery, and biological significance of chlorophyll loss in *Rosmarinus officinalis* plants. *Planta*, **210**: 925-931.

Oquiset, G., Campbell, D., Clarke, A. K. and Gustafsson, P. (1995). The cyanobacterium *Synechococcus* modulates photosystem II

function in response to excitation stress through D1 exchange. *Photosynthesis Research,* **46:** 151-158.

Schreiber, U., Bilger, W., Hormann, H. and Neubauer, C. (1998). Chlorophyll fluorescence as a diagnostic tool: Basics and some aspects of practical relevance. In: *Photosynthesis: A Comprehensive Treaties,* (Ed. A. S. Raghavendra), Cambridge University Press, U. K., pp. 320-336.

Suzuki, T. C., Krawitz, D. C. and Vierling, E. (1998). The chloroplast small heat shock protein oligomer is not phosporylated and does not dissociate during heat stress *in vivo. Plant Physiology,* **116**: 1151– 1161.

Torok, Z., Goloubinof, P., Horvath, I., Tsvetkova, N. M., Glatz, A., Balogh, G., Varavasovski, V., Los, D. A., Vierling, E., Crowe, J. H. and Vigh, L. (2001). *Synechocystis* HSP 17 as an amphitropic protein that stabilize heat stressed membranes and binds denatured proteins for subsequent chaperone mediated refolding. *Proceedings of the National Academy of Sciences,* USA, **98**: 3098-3103.

Vierling, E. (1991). The role of heat shock protein in plants. *Annual Review of Plant Physiology and Plant Molecular Biology,* **42**: 579-620.

Walker, D.A. (1988). *The use of oxygen electrode and fluorescence probe in simple measurements of photosynthesis.* II ed. (Oxygraphics Ltd. Sheffield), pp. 57-69.

Wehmeyer, N., Hernandez, L. D., Finkelstein, R. R. and Vierling, E. (1996). Synthesis of small heat shock protein is part of the development program of late seed maturation. *Plant Physiology,* **112**: 747-757.