Research Article

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Drought tolerance in MnSOD transgenic *Hevea brasiliensis* in a dry sub-humid environment

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Abstract

One year old bud-grafted plants of MnSOD transgenic *Hevea* lines (L1 and L2) and an untransformed line of clone RRII 105 were used in the present study to evaluate their physiological performance in a dry sub-humid environment by withholding irrigation and to assess the recovery by re-watering. The dry matter partitioning was more towards the root in transgenic lines (55% and 60% in Ll and L2, respectively) but, was less in the untransformed RRII 105 (43%). After six days of moisture stress in polybags, pre-dawn leaf water potential and relative water content declined in all the lines, however, transgenic line L1 showed higher tissue water content throughout the drought as well as recovery period. Chlorophyll content did not show any significant reduction. Net photosynthetic rate (P_N) declined rapidly and it reached near zero on the third day of drought imposition except for line L1, which showed lesser decline in P_N. The decline in stomatal conductance (g_s) was more rapid than P_N in all the lines. On re-watering, recovery of P_N and g_s was better in the transgenic lines than untransformed RRII 105, which did not recover fully from the drought impact. Antioxidant enzymes, superoxide dismutase and peroxidase did not show a definite trend in their activities in these lines. However, it was found that the transgenic line L1 had better drought tolerant capacity in terms of lesser inhibition of photosynthetic rate under drought and faster recovery on re-watering.

Keywords: Drought, *Hevea brasiliensis*, MnSOD, oxidative stress, transgenic plants

Introduction

The production and productivity of natural rubber (*Hevea brasiliensis*) has to be increased further to meet the world wide growing demands of rubber based industry. Developing high yielding rubber clones that come up well under the changing climatic conditions is the only option to achieve this goal by improving productivity. Clones with increased tolerance to biotic and abiotic stresses are the need of the hour, as increasing the acerage of the crop will be difficult for want of suitable land.

Drought or water stress causes physiological changes such as decrease in leaf water potential, photosynthetic rate, stomatal closure, *etc.* which results in the production of reactive oxygen species (ROS) and inhibition of antioxidant systems in plants (Mittler and Zilinskas, 1994). Of the antioxidant enzymes, superoxide dismutase (SOD) activity forms the first line of defence against ROS. SOD is a family of metallo-enzymes which are known to accelerate the spontaneous dismutation of superoxide radical (O_2^-) to hydrogen peroxide (H_2O_2). Depending on the metal co-factor used by the enzyme, SODs are classified into three groups: copper-zinc SOD (Cu/ZnSOD), manganese SOD (MnSOD) and iron SOD (FeSOD). Cu/ZnSODs are cytosolic, chloroplastic or peroxisomal isoforms, MnSODs are mitochondrial or peroxisomal isoforms and FeSODs are chloroplastic isoforms in nature (Leclercq *et al.,* 2012).

Under the present scenario of rising temperature due to climate change and depleting

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ground water levels, development of genetically modified crops has come as a major tool to address the harmful environmental. Different genes have been successfully transferred into several crop plants worldwide and over-expression of such genes resulted in increased protection against biotic and abiotic stresses (Gao *et al*., 2003; Prashanth *et al*., 2008; Wang *et al*., 2010; Faize *et al*., 2011). Survival of transgenic plants significantly improved in field trials with sustainable yield, supporting the hypothesis that tolerance to oxidative stress is important in adaptation to adverse environments (McKersie *et al*., 1996, 1999). Such studies give an indication that over-expression of antioxidant genes improves the stress tolerance potential of economically important plants.

In an attempt to impart tolerance to oxidative stress, the Rubber Research Institute of India (RRII) has developed genetically modified *Hevea* plants by over-expressing MnSOD gene through *Agrobacterium* mediated genetic transformation in clone RRII 105 (Jayashree *et al*., 2003). The objective of the present study was to assess the physiological performance of MnSOD transgenic lines (L1 and L2) and an untransformed control plants of RRII 105 for their tolerance to drought under water deficit conditions in the North Konkan region of Maharashtra state in India.

Materials and methods

Plant material and location

One year old bud grafted plants of untransformed RRII 105 and MnSOD transgenic lines (L1 and L2) developed at RRII, Kottayam were used in this study. The plants were grown in polybag containers filled with garden soil in a protected area under natural conditions at Regional Research Station, Dapchari, Maharashtra situated at 20°04′N and 72°04′E with an elevation of 48 m above MSL. The incident solar and UV-B radiations in the location were recorded diurnally at hourly intervals using a photo-radiometer, PMA 2200 (Solar Light Co., USA). During the summer months (May-June) of 2012, one set of plants (n=6) were irrigated daily up to field capacity and another set of equal number of plants was kept un-irrigated for six days. After sixth day of drought treatment, the plants were irrigated daily to study the extent of drought recovery. Gas exchange and water potential measurements in irrigated and water stressed plants were done daily from the first day of withholding irrigation, while, chlorophyll content index and relative water content (RWC) of leaves were measured on alternate days of drought period. Leaf samples were collected from a different set of same plants on the third day of drought treatment, preserved in dry ice and transported to RRII laboratory, Kottayam for biochemical analysis.

Biomass partitioning and plant-water relations

Biomass partitioning in these lines were studied by gravimetric measurements, taking 10 plants randomly from each line and the dry weight of leaves, petiole, stem, taproot and lateral roots were recorded individually. Pre-dawn leaf water potential (ψ_L) was measured at 6.00 am using a dew point micro-voltmeter connected with C-52 sample chambers using Psypro water potential system (Wescor, USA). The leaf discs obtained by a paper punch were put in the C-52 sample chamber. After an equilibration period of 30 minutes, readings were taken and the temperature correction of the values was done using the correction factor. RWC was determined by taking the fresh weight, turgid weight and dry weight of leaf bits according to Barrs and Weatherley (1962). RWC was calculated from the following equation:

RWC = [(fresh weight - dry weight) / (turgid weight - dry weight)] x 100

Gas exchange measurements

Gas-exchange parameters were measured on fully mature leaves in the top whorl using a Li-6400XT portable photosynthesis system (Li-Cor, Lincoln, USA). The net photosynthetic rate (P_N), and stomatal conductance (g_s) were recorded on sunny days (08:00 to 10:00 hrs IST) at a light intensity of 500 µmol m^{-2} s^{-1} provided by red and blue light using a leaf chamber fluorometer attached to the system, maintaining the CO_2 concentration at 400 µmol m^{-2} s^{-1} using a CO_2 injector (Alam *et al*., 2005). The leaf chlorophyll content index (CCI) was determined with a chlorophyll meter (SPAD-502, Minolta Inc., Japan) on mature, fully expanded leaves.

Biochemical analysis

Leaf samples were collected from each treatment and triplicate samples were used for enzyme assays and determining the lipid peroxidation. Total superoxide dismutase (SOD) and peroxidase enzymes were extracted from leaf tissues by homogenizing with 50 mM phosphate buffer pH 7.4. The extracts were centrifuged at 12,000 rpm for 20 min at 4 °C. Total SOD activity was assayed by the method described by Giannopolitis and Ries (1977). One unit of SOD was defined as the amount of enzyme that produced 50 per cent reduction of nitroblue-tetrazolium (NBT) under the assay conditions. Peroxidase activity was determined according to the method of Guilbault (1977). One unit of enzyme was defined as the change in optical density (OD) $min^{-1}mg^{-1}$ protein. Lipid peroxidation was estimated by determining the malondialdehyde (MDA) content in the leaf according to Heath and Packer (1968). Leaf samples were homogenized in 0.1 per cent trichloroacetic acid (TCA) and the homogenate was centrifuged at 12,000 rpm for 20 min at 4 °C. About 0.3 ml of supernatant was mixed with 1.2 ml of 0.5 per cent thiobarbituric acid prepared in 20 per cent TCA and incubated at 95 °C for one hour. After stopping the reaction in an ice bath, the optical density was read at 532 nm and 600 nm. After subtracting the non-specific absorbance at 600 nm, MDA concentration was determined using the extinction coefficient 155 mM^{-1} cm^{-1}.

Data presented are mean of 4-6 measurements and were subjected to analysis of variance (ANOVA). Cropstat V7.2 was used for the statistical analysis of data.

Results and discussion

The experiment was conducted during peak summer season which was highly stressful to rubber plants with soil moisture deficit, high temperature, low humidity and solar radiation exceeding 2000 µmol m^{-2} s^{-1} around midday (Fig. 1). The incident UV-B radiation was also on the higher side crossing 4.2 Wm^{-2} which is a matter of concern in this region. The maximum and minimum temperatures during the study period were 35-37 °C and 24-28 °C respectively; relative humidity varied from 77 per cent in the morning to 50 per cent in the afternoon and the average sunshine hours was 10 hrs.

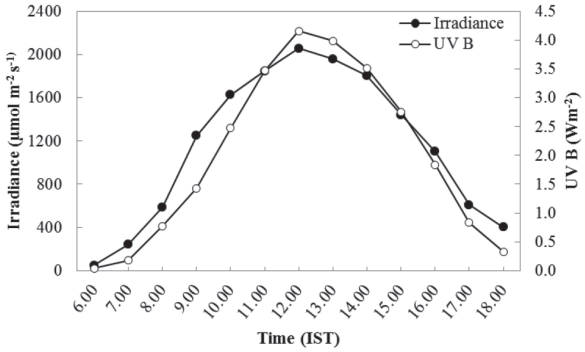

Fig. 1. Diurnal solar and UV-B radiations during a typical summer period at Dapchari in Maharashtra in 2012

More dry matter in roots of transgenic plants

Both the transgenic lines (L1 and L2) showed significantly high dry matter partitioning towards the taproot. Partitioning to other parts of the plants was similar in all the lines (Fig. 2). Dry matter accumulation in the root system was 55 and 60 per cent in lines L1 and L2 respectively, while it was only 43 per cent in untransformed RRII 105. In other words, the root:shoot ratio was 0.75 for untransformed line, while it was 1.2 and 1.5 for L1 and L2, respectively. Under well irrigated condition the root:shoot ratio in transgenic plants was higher than untransformed RRII 105. During soil moisture deficit condition, plants try to obtain water from deep layers of soil, by enhancing its root system which is a general adaptation strategy under stress (Xu *et al.*, 2010). Increased biomass accumulation

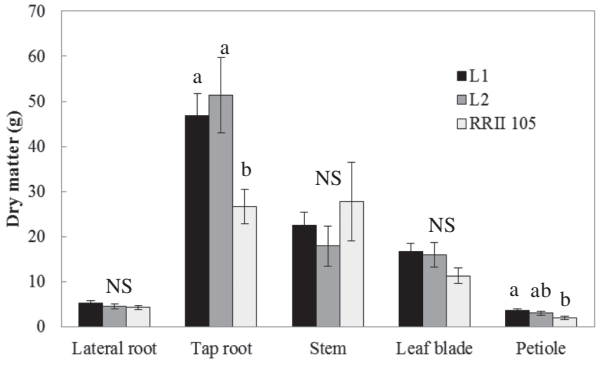

Fig. 2. Dry matter partitioning in different plant parts of the transgenic lines (L1 and L2) and untransformed RRII 105. Bars with same letter are not significantly different from each other, (P≤0.05) NS- non significant

in the root under dry environments acts as a defensive mechanism to overcome drought stress. However, in the present study, since the rootstock is raised from polycross seeds, it needs to be studied whether the increased biomass allocation to root is an inherent character of stock, or influenced by genetic modification of scion.

Water relations in transgenic lines under drought

Pre-dawn water potential showed a gradual decline upon imposition of water deficit stress for the initial three days and thereafter the rate of decline was fast in all the lines (Fig. 3). Transgenic

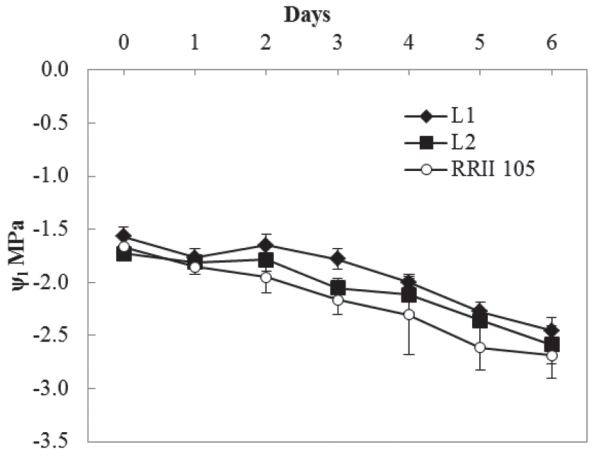

Fig. 3. Pre dawn leaf water potential values of the transgenic lines (L1 and L2) and untransformed RRII 105 during 6 days of moisture stress

line L1 maintained better ψ_L from the second day of drought imposition till sixth day of moisture stress. Decline in water potential was more in the untransformed plants. However, after third day of re-watering, all the lines showed recovery of water potential to the initial level. Drought stress reduced RWC in all the lines. RWC was in the range of 84 to 87 per cent in the irrigated plants and after six days of moisture stress the water content in the leaves reduced to 62-68 per cent indicating moderate to severe stress (Fig. 4). Decline in RWC was comparatively lesser in L1 (22%) than L2 (25%) and RRII 105 (27%). In plants, a decrease in ψ_L can be an indication of decrease in available soil moisture or incidence of drought. Under moderate

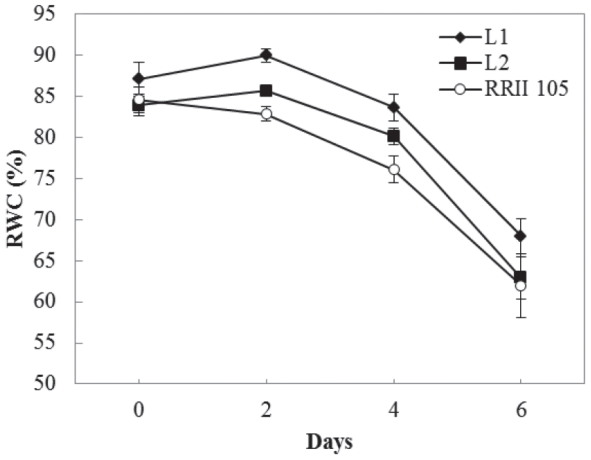

Fig. 4. Relative water content of the transgenic lines (L1 and L2) and untransformed RRII 105 at 0, 2, 4 and 6 days of moisture stres

moisture stress, tissue water potential and water content are maintained close to the unstressed level, either by increasing the water uptake or limiting the water loss, so that, the rates of water uptake and water loss remain balanced (Verslues *et al.,* 2006). In the present study, the transgenic plants showed a certain degree of stable ψ_L and RWC on the second day of drought treatment which can be a short term adjustment to protect the water loss by stomatal closure or by increased antioxidant scavenging. When the drought progressed, a steady decline in water content in the plant was noticed. The tissue water status under drought was significantly higher in the transgenic lines L1 and L2 than untransformed control.

Variation in chlorophyll content

Chlorophyll content index (SPAD) declined slightly in all the lines upon drought treatment. Though transgenic plants L2 had higher chlorophyll content throughout the study, there was no significant variation in chlorophyll index among the lines studied (Fig. 5). CCI is a non-destructive and easy field level assay of leaf health. Significant correlation was derived between CCI and actual chlorophyll content in *Hevea* (Nair and Jacob, 2011). Smaller reduction in mid-day leaf water potential, effective quantum yield of PS II and photosynthetic oxygen evolution in transgenic line L1 were reported after withholding irrigation for two weeks in the traditional region (Jayashree *et al*.,

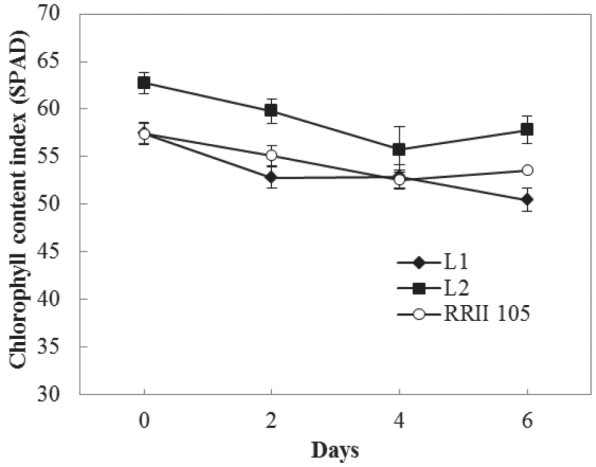

Fig. 5. Chlorophyll content index of the transgenic lines (L1 and L2) and untransformed RRII 105 at 0, 2, 4 and 6 days of moisture stress

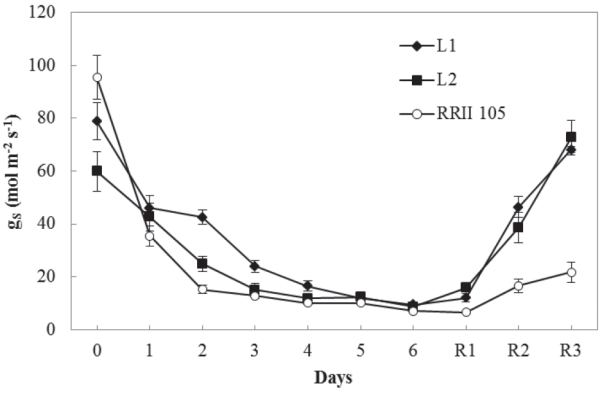

Fig. 7. Stomatal conductance (g_s) in the transgenic lines (L1 and L2) and untransformed RRII 105 during six days of drought stress and recovery after re-watering for 1-3 days (R_1-R_3)

2011). In the present study, the plants showed symptoms of drying after six days of moisture stress, indicating the severity of the stress aggravated by high temperature, high light and high vapour pressure deficit mediated atmospheric drought. The rapid development of drought may be the reason for the low response obtained in chlorophyll content, which take longer time for responding to stress condition, unlike gas exchange parameters.

Recovery of CO_2 assimilation and stomatal conductance

A progressive decline was observed in CO_2 assimilation with water stress. Under irrigated condition, untransformed RRII 105 had the highest P_N compared to the transgenic lines (Fig. 6). Photosynthetic rate reached near zero after three days of moisture stress in L2 and RRII 105; whereas the rate of decline in P_N was slow in L1 and reached zero only on the fifth day of imposing moisture stress. After six days of water stress, re-watering mediated recovery was better in the transgenic lines, which reached near control values in transgenic plants after three days of re-watering. The untransformed RRII 105 showed a slow rate of recovery and the P_N recovered only 27 per cent after re-watering for three days. Similarly, the stomatal conductance (g_s) also showed a decline under moisture stress (Fig. 7) and the decline was less in L1 compared to L2. After 5 days of moisture stress, g_s reached near zero and the rate of decline was high in RRII 105. On re-watering, the transgenic lines showed better recovery rates of g_s compared to the untransformed RRII 105 plants.

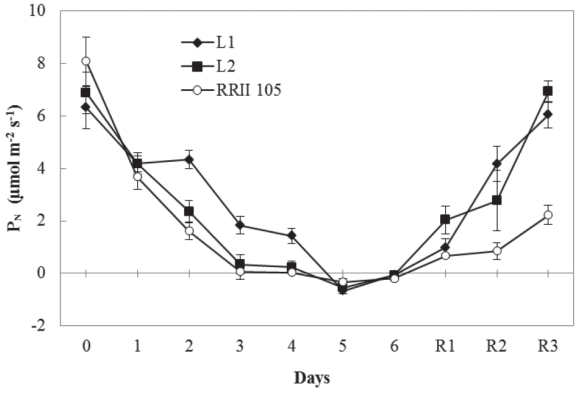

Fig. 6. Photosynthetic rate (P_N) of the transgenic lines (L1 and L2) and untransformed RRII 105 during six days of drought stress and recovery after re-watering for 1-3 days (R_1-R_3)

Decline in photosynthesis under drought stress is well documented in plants. A combination of water stress, high temperature, increased atmospheric VPD, nutrient depletion, high irradiance, *etc*. can limit the photosynthetic capacity of plants (Flexas *et al*., 1999). Decreased CO_2 diffusion from the atmosphere to the site of carboxylation is generally considered as the main cause for decreased photosynthesis under mild to moderate water limitation (Flexas *et al*., 2004; Grassi and Magnani, 2005; Chaves *et al*., 2009). In

Table-1. Antioxidant enzyme activities and lipid peroxidation in irrigated and drought stressed (3 days) plants of transgenic lines (L1 and L2) and untransformed RRII 105

Line	SOD (units)		Peroxidase (units)		MDA (μmol g^{-1} fw^{-1})	
	Control	Drought	Control	Drought	Control	Drought
L1	3.12±0.39	2.58±0.24	0.84±0.50	0.89±0.05	2.12±0.40	3.20±0.80
L2	5.38±0.39	6.17±0.71	0.52±0.04	0.28±0.03	2.84±0.23	5.31±1.01
RRII 105	3.63±0.36	2.88±0.50	0.33±0.03	0.77±0.05	1.56±0.05	1.84±0.20

± Standard error (n=5)

transgenic tobacco and potatao, over-expression of chloroplast Cu/Zn SOD showed improvement in the photosynthetic performance under chilling stress (Sen Gupta *et al*., 1993; Perl *et al*., 1993). Full recovery of net photosynthetic rate has been observed while withdrawing the drought stress by re-watering in perennial grass ecosystem. It has been shown that re-watering almost completely nullified the difference between drought-treated and the control plants, which showed significant drop in light saturated net photosynthetic rate (Xu *et al*., 2009). Stomatal closure is an immediate response of the plant to soil moisture stress. In the present study, g_s reduced by 42 per cent in L1, 30 per cent in L2 and more than 62 per cent in untransformed RRII 105 in just 24 hours of withholding irrigation. Stomatal closure reduces the availability of CO_2 for carbon reduction, leading to the formation of ROS owing to excess electrons generated in the light reactions. Therefore, scavenging mechanisms that reduce the level of oxidative stress may be an initial step in plants to overcome drought effects.

Antioxidant defense

Increased SOD activity was observed in the transgenic line L2 under irrigated and drought conditions, whereas, L1 and untransformed RRII 105 showed comparable activity (Table 1) under drought condition. Peroxidase activity was, in general, low in irrigated and drought imposed transgenic plants but it increased in untransformed RRII 105 under drought condition. In the transgenic line L1, drought treatment did not induce any change in the level of peroxidase activity, and there was a decline in peroxidase activity in line L2. Lipid peroxidation was comparatively more in line L2 (5.31 μmol MDA g^{-1} fw^{-1}) than L1 (3.20 μmol MDA g^{-1} fw^{-1}) and in untransformed RRII 105 (1.84 μmol MDA g^{-1} fw^{-1}) under drought condition.

The assay of antioxidant scavenging enzyme activities in the leaves of transformed and untransformed plants showed variations, three days after drought treatment. Similar to our observations, Leclercq *et al.* (2012) did not find any over activity of SOD in relation with over-expression of *HbCuZnSOD* gene in *Hevea*. They also noted varied activity of antioxidant ezymes under water deficit in the transgenic lines though the relative transcript abundance was stable and reproducible. Increased lipid peroxidation in transgenic lines can be due to increased H_2O_2 accumulated, which were not effectively scavenged by the peroxidase in the plant system. Slooten *et al*. (1995) reported tobacco transgenic plants over-expressing MnSOD rendered enhanced tolerance to oxidative stress only in the presence of other antioxidant enzymes and substrates, highlighting that the genotype and the isozyme composition also have a profound effect on the relative tolerance of the transgenic plants to abiotic stresses. Faulty function of the antioxidant gene products, the physiological targets studied, the severity of the stresses imposed, and/or the plant systems used, are also attributed for the low response of transgenic plants to a specific stress situation for which they were developed (Van Camp *et al*., 1996). Bowler *et al*. (1991) reported sub-cellular locations like chloroplastic, mitochondrial, cytoplasmic *etc.* are likely to play a major role in determining effects, whether positive or negative, as a result of SOD overproduction.

Conclusion

The physiological performance of transgenic lines was better than the untransformed RRII 105 in terms of water relations and photosynthetic parameters during drought and drought recovery period. Among the transgenic plants, line L1 was found relatively better performing under drought conditions. However, the drought tolerance

potential of transgenic plants should be further ascertained in actual field conditions.

References

Alam, B., Nair, D.B. and Jacob, J. 2005. Low temperature stress modifies the photochemical efficiency of a tropical tree species *Hevea brasiliensis:* Effects of varying concentrations of CO_2 and photon flux density. *Photosynthetica* **43**(2): 247-252.

Barrs, H.D. and Weatherley, P.E. 1962. A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Australian Journal of Biological Sciences* **24**: 519-570.

Bowler, C., Slooten, L., Vandenbranden, S., Rycke, R.D., Botterman, J., Sybesma, C., Montagu, M.V. and Inze, D. 1991. Manganese superoxide dismutase can reduce cellular damage mediated by oxygen radicals in transgenic plants. *The EMBO Journal* **10**(7): 1723-1732.

Chaves, M.M., Flexas, J. and Pinheiro, C. 2009. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Annals of Botany* **103**: 551-560.

Faize, M., Burgos, L., Faize, L., Piqueras, A., Nicolas, E., Barba-Espin, G., Clemente-Moreno, M.J., Alcobendas, R., Artlip, T. and Hernandez, J.A. 2011. Involvement of cytosolic ascorbate peroxidase and Cu/Zn-superoxide dismutase for improved tolerance against drought stress. *Journal of Experimental Botany* doi:10.1093/jxb/erq432.

Flexas, J., Bota, J., Loreto, F., Cornic, G. and Sharkey, T.D. 2004. Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants. *Plant Biology* **6**: 269-279.

Flexas, J., Escalona, J.M. and Medrano, H. 1999. Water stress induces different levels of photosynthesis and electron transport rate regulation in grapevines. *Plant Cell and Environment* **22**: 39-48.

Gao, X., Ren, Z., Zhao, Y. and Zhang, H. 2003. Overexpression of SOD2 increases salt tolerance of Arabidopsis. *Plant Physiology* **133**(4): 1873-1881.

Giannopolitis, C.N. and Ries, S.K. (1977). Superoxide dismutase: Occurrence in higher plants. *Plant Physiology* **59**: 309-314.

Grassi, G. and Magnani, F. 2005. Stomatal, mesophyll conductance and biochemical limitations to photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. *Plant, Cell and Environment* **28**: 834-849.

Guilbault, G.G. 1976. *Handbook of Enzymatic Methods of Analysis*, Maracel Dekker Inc. 147p.

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**: 189-198.

Jayashree, R., Rekha, K., Venkatachalam, P., Uratsu, S.L., Dendekar, A.M., Jayasree, P.K., Kala, R.G., Priya, P., Sushamakumari, S., Sobha, S., Asokan, M.P., Sethuraj, M.R. and Thulaseedharan, A. 2003. Genetic transformation and regeneration of rubber tree (*Hevea brasiliensis* Muell. Arg) transgenic plants with a constitutive version of an anti-oxidative stress superoxide dismutase gene. *Plant Cell Report* **22**: 201-209.

Jayashree, R., Sobha, S., Rekha, K., Supriya, R., Vineetha, M., Sushamakumari, S., Kala, R.G., Jayasree, P.K., Thulaseedharan, A., Annamalainathan, K., Nair, D.B., Sreelatha, S., Krishnakumar, R. and Jacob, J. 2011. Over-expression of MnSOD and related drought tolerant traits in MnSOD transgenic *Hevea brasiliensis*. *Natural Rubber Research* **24**(1): 18-27.

Leclercq, J., Martin, F., Sanier, C., Cle´ment-Vidal, A., Fabre, D., Oliver, G., Lardet, L., Ayar, A., Peyramard, M. and Montoro, P. 2012. Over-expression of a cytosolic isoform of the HbCuZnSOD gene in *Hevea brasiliensis* changes its response to a water deficit. *Plant Molecular Biology* **80**: 255-272.

McKersie, B.D., Bowley, S.R. and Jones, K.S. 1999. Winter survival of transgenic alfalfa overexpressing superoxide dismustase. *Plant Physiology* **119**: 839-847.

McKersie, B.D., Bowley, S.R., Harjanto, E. and Leprince, O. 1996. Water deficit tolerance and field performance of transgenic alfalfa overexpressing superoxide dismutase. *Plant Physiology* **111**: 1177-1181.

Mittler, R. and Zilinskas, B.A. 1994. Regulation of pea cytosolic ascorbate peroxidase and other antioxidant enzymes during the progression of drought stress and following recovery from drought. *The Plant Journal* **5**(3): 397-405.

Nair, D.B. and Jacob, J. 2011. A simple method for large scale estimation of leaf chlorophyll content in *Hevea brasiliensis* using chlorophyll meter. *NBU Journal of Plant Sciences* **5**(1): 47-49.

Perl, A., Perl-Treves, R., Galili, S., Aviv, D., Shalgi, E., Malkin, S. and Galun, E. 1993. Enhanced oxidative stress defense in transgenic potato expressing tomato Cu, Zn superoxide dismutases. *Theoretical and Applied Genetics* **85**(5): 568-576.

Prashanth, S.R., Sadhasivam, V. and Parida, A. 2008. Over expression of cytosolic copper/zinc superoxide dismutase from a mangrove plant *Avicennia marina* in *indica* rice var Pusa Basmati-1 confers abiotic stress tolerance. *Transgenic Research* **17**(2): 281-291.

Sen Gupta, A., Heinen, J.L., Holady, A.S., Burke, J.J. and

Allen, R.D. 1993. Increased resistance to oxidative stress in transgenic plants that over-express chloroplastic Cu/Zn superoxide dismutase. *Proceedings of the National Academy of Sciences* **90**: 1629-1633.

Slooten, L., Capiau, K., Van Camp, W., Montagu, M.V., Sybesma, C. and Inze´, D. 1995. Factors affecting the enhancement of oxidative stress tolerance in transgenic tobacco overexpressing manganese superoxide dismutase in the chloroplasts. *Plant Physiology* **107**: 737-775.

Van Camp, W., Capiau, K., Montagu, M.V., Inzé, D. and Slooten, L. 1996. Enhancement of oxidative stress tolerance in transgenic tobacco plants overproducing Fe-superoxide dismutase in chloroplasts, *Plant Physiology* **112**(4): 1703-1714.

Verslues, P.E., Agarwal, M., Katiyar-Agarwal, S., Zhu, J. and Zhu, J-K. 2006. Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. *The Plant Journal* **45**: 523-539.

Wang, Y.C., Qu, G.Z., Li, H.Y., Wu, Y.J., Wang, C., Liu, G.F. and Yang, C.P. 2010. Enhanced salt tolerance of transgenic poplar plants expressing a manganese superoxide dismutase from *Tamarix androssowii*. *Molecular Biology Reports* **37**(2): 1119-1124.

Xu, Z.Z., Zhou, G.S. and Shimizu, H. 2009. Are plant growth and photosynthesis limited by pre-drought following rewatering in grass? *Journal of Experimental Botany* **60**: 37-49.

Xu, Z.Z., Zhou, G.S. and Shimizu, H. 2010. Plant responses to drought and re-watering. *Plant Signaling and Behavior* **5**(6): 649-654.