

Comparison of Tolerant Ability of Mature Field Grown Tea (*Camellia sinensis* L.) Cultivars Exposed to a Drought Stress in Passara Area

M.M.N. Damayanthi, A.J. Mohotti^{1*} and S.P. Nissanka¹

Agronomy Division
Tea Research Institute, Talawakele
Sri Lanka

ABSTRACT. *The response of some selected physiological and biochemical parameters of tea (*Camellia sinensis* L.) plants to moisture stress was evaluated using field grown, mature plants, in Field Gene Bank at Passara, Sri Lanka, to identify traits that are associated with drought tolerance which could be used for cultivar screening programs. This study was carried out from July - October 2008. Known contrasting cultivars in terms of drought tolerance were selected from the existing gene bank for the measurements. Physiological and biochemical parameters were measured to study the response of tea plants to drought stress. Identification of traits was done by using cluster analysis, cultivar × environment interaction and drought susceptibility index. Reduction of leaf water potential up to -0.75 to -1.5 MPa at the end of the dry period indicated that the plants were exposed to a sufficient level of moisture stress. The drought tolerant cultivars maintained a high water status with substantial rate of photosynthesis as a result of osmotic adjustments, elevated total soluble sugars and also according to previous research, due to deep rooting. Drought susceptible cultivars maintained a lower water status with lower osmotic adjustment. With cluster analysis using rates of photosynthesis and transpiration, relative water content, stomatal conductance and leaf total soluble sugar content, the cultivars could be clearly grouped as tolerant and susceptible cultivars, which agreed with their tolerance level. The parameters which could be successfully incorporated into a drought screening procedure were rate of photosynthesis, stomatal conductance, transpiration rate, relative water content and total soluble sugar content.*

Key words: *Cultivar differences, Drought, Physiological and biochemical responses, Tea (*Camellia sinensis* L.).*

INTRODUCTION

Tea (*Camellia sinensis* L.), a small, evergreen, woody perennial tree is one of the most important beverage crops in the world. Tea industry in Sri Lanka is the main net foreign exchange earner and the country's largest employment provider that directly and indirectly provides employment for over one million people. Tea is grown primarily as a rain fed plantation crop and tea yield is greatly influenced by weather. Although monsoonal rains bring sufficient amount of rainfall, irregular distribution within a year causes moisture stress on tea plants mainly during the months of January, March and August resulting in a

* To whom correspondence should be addressed to: mohotti@yahoo.com

¹ Department of Crop Science, Faculty of Agriculture, University of Peradeniya, Peradeniya

considerable loss of crop and increased casualties (Karunaratne *et al.*, 1999). Therefore, drought is one of the major constraints to improve tea productivity in certain parts of Sri Lanka.

Drought stress brings about many physiological, biochemical and morphological changes in plants. Water stress is known to cause a reduction of leaf water potential, photosynthesis and stomatal conductance (Bota *et al.*, 2004). Proline and abscisic acid (ABA) accumulate in higher concentrations in many tolerant species in response to water stress, which leads to maintenance of turgour potential in plants (Morgan, 1984). Moreover, water stress increases biomass partitioning to roots resulting in increased root dry weights, root length density and root: shoot ratio (De Costa, 2004).

This study was conducted to identify the physiological and biochemical parameters that could best reflect the drought effects in different cultivars under field conditions in a drought prone area of Sri Lanka, with a view to incorporating them into a screening procedure of drought tolerance, to check whether these cultivars could be separated into drought tolerant and susceptible groups based on these characters and whether this grouping agrees with the available information regarding the drought tolerance of cultivars.

MATERIALS AND METHODS

Experimental site

This experiment was conducted at the Field Gene Bank at the Tea Research Institute, Passara substation at an elevation of 1120 m average mean sea level. This area normally experiences seasonal dry spells during the months of July to September. A prolonged dry period was experienced from July- October 2008. Eight cultivars were selected from the existing gene bank for the measurements, based on the understanding of their drought tolerance ability, i.e. either drought tolerant (TRI 2025, DN, CY9, DG 7 and DG 39) or susceptible (TRI 2023, TRI 2024, TRI 2026) (Anonymous, 2002). There were 78 cultivars arranged in plots of 25 plants in three rows with two replicates. The measurements were carried out after ceasing of rains in August 2008 and continued until the rains commenced in October 2008.

Plants and soil moisture content (SMC) were measured in 12 representative samples selected randomly from each treatment. Changes in physiological and biochemical parameters over time were studied during this period. For the physiological and biochemical measurements, mature, fully grown, healthy leaves on the plucking table were selected.

Measurements

The following physiological and biochemical parameters along with soil moisture content were measured to study the responses of tea plants to drought. Measurements were made from 9.00 a.m. to 12.30 p.m.

Soil moisture content (SMC)

Soil moisture content of the root zone at 25 cm depth was measured along with physiological and biochemical parameters from 9.00 am to 12.30 pm using Theta probe soil moisture meter (Soil moisture sensor, Delta-T Devices Ltd., UK).

Gas exchange parameters

The gas exchange parameters were measured in the most recently fully matured leaves. A portable photosynthesis system (model: LI- 6200, Li-Cor Inc., USA) was used to determine the net photosynthetic rate (P), stomatal conductance (Gs) and transpiration (TP).

Relative water content (RWC)

Relative water content of leaves was determined according to the method explained by Wijerathne (1996).

Leaf water potential (LWP)

Mid-day leaf water potential was measured on the entire leaf by observing the presence of water on the cut surface of the leaf petiole using a pressure chamber (SKPM 1400, SKYE Instruments, UK).

Stomatal density (SD)

Stomatal density was measured in young fully matured mother leaves using the stomatal impressions of intact leaves. These were taken by application of a thin layer of nail varnish on the lower leaf surface, allowing it to dry, peeling it with a clear scotch tape and observing under a light microscope (LEICA DMLS) on a glass microscopic slide. The measurements were made using an eye piece graticule and a stage micrometer (LX # 68010-21, 01A21001, Graticules Ltd, England).

Leaf chlorophyll content

Leaf discs of 10 cm² were collected into labeled aluminium foil bags and immediately stored in ice to avoid formation of polyphenolic compounds at the damaged surface, and stored at -84°C until analysis. Chlorophyll contents were assayed as per the method described by Arnon (1949).

Total soluble sugar content (TSS)

For sugar determination, leaf discs of known areas were cut and placed in a solution containing 600 mL methanol, 250 mL Chloroform and 150 mL distilled water and stored at -84° C until extraction. The samples were assayed according to Mohotti and Lawlor (2002).

Drought susceptibility index

The drought susceptibility index (DSI) proposed by Fischer and Maurer (1978) was calculated using photosynthesis rate as a reference parameter (Nainanayake, 2004). The following formula was used for the calculation.

$$DSI = [1-Y/Y_p] / [1-X/X_p]$$

Where, Y_p = photosynthesis under no stress, Y = photosynthesis under stress, X = average photosynthesis over all genotypes under stressed conditions, and X_p = average photosynthesis over all genotypes under non-stressed conditions.

Statistical analysis

Statistical analysis was performed using ANOVA and mean separation by Duncan New Multiple Range Test using the SAS statistical package. Identification of parameters that are most suitable for screening was by using cluster analysis, cultivar- environment interaction and drought susceptibility index.

RESULTS AND DISCUSSION

This field experiment was conducted to identify responses of some commonly grown tea cultivars to drought in field grown plants in Passara area. Changes in the measured physiological and biochemical parameters over time as the drought proceeded were studied during July- October 2008.

Table 1. Changes in mean soil moisture contents of different cultivars

Cultivar	Average soil moisture content % (v/v) ¹				
	Day 1	Day 2	Day 3	Day 4	Day 5
TRI 2025	13.7	12.5	9.6	8.8	8.0
DN	15.7	12.5	9.3	9.0	8.3
CY9	11.4	10.1	9.5	7.9	7.8
DG7	12.8	12.2	10.3	9.3	8.4
DG39	13.1	12.3	10.2	8.7	8.2
TRI 2024	12.7	10.3	9.0	8.1	8.0
TRI 2026	11.0	9.9	6.7	7.8	6.3
TRI 2023	11.4	10.6	10.2	7.1	7.5
Average	12.7	11.3	9.4	8.3	7.8
CV	30.3	17.6	35.3	35.7	28.5

Day 1-5 denotes different days of measurement (approximately 18, 35, 42, 49, 83 days after the rain stopped).

¹v/v- Volume in volume.

The mean soil moisture content averaged across all varieties was 12.72, 11.29, 9.36, 8.33 and 7.79% (v/v) on daily 1, 2, 3, 4 and 5, respectively (Table 1). Days 1-5 denotes different days of measurement (18, 35, 42, 49, 83 days after the rains stopped). The lowest soil moisture content (7.79%) was attained approximately 83 days after rains stopped.

Average soil moisture content fluctuated within a narrow range across varieties at a given day of measurement. Soil moisture content in the root zone of different varieties during the drought period was not significantly ($p < 0.05$) different. The changes in leaf water potential (LWP) and relative water content (RWC) of different cultivars are presented in Fig. 1a and b, respectively.

The mean LWP was significantly (< 0.05) different amongst cultivars and different soil moisture levels. The cultivar \times moisture level interaction was also significant ($P < 0.05$). At the lowest moisture levels, drought resistant cultivars such as DN, TRI 2025, CY9 maintained a significantly higher ($P < 0.05$) LWP compared to the drought susceptible cultivars (TRI 2026, TRI 2023).

The mean leaf RWC were significantly ($P < 0.05$) different amongst cultivars and amongst different soil moisture contents. The interaction between cultivar and moisture level was also significant ($P < 0.05$).

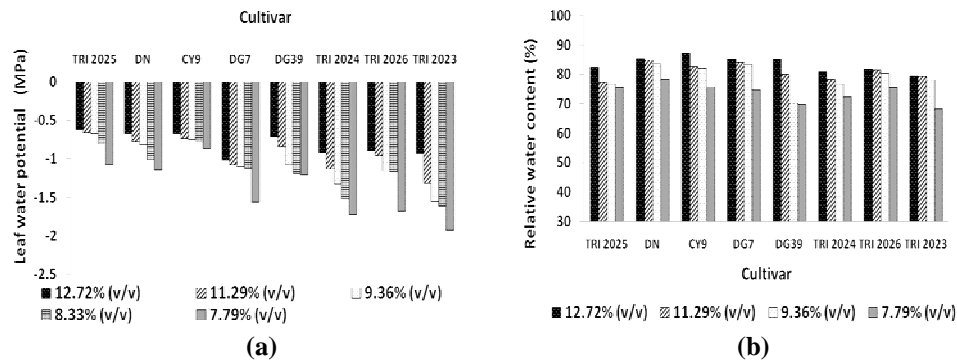


Fig. 1. Variation in (a) leaf water potential and (b) relative water content of different cultivars with gradual decrease in soil moisture level.

With the initially high soil moisture content, RWC did not vary significantly among cultivars. With the depletion of soil moisture content, LWP and RWC declined rapidly. Some cultivars maintained a high LWP (TRI 2025, DN and CY9, which were categorized as drought tolerant) even at very low soil moisture levels compared to the other cultivars. These cultivars maintained LWP around -1MPa when most of the other cultivars showed lower LWP (-1.5 to -2 MPa). Losses of tissue water lead to reduction of activity of cellular water, thereby the chemical potential, reduction of turgor pressure, increased concentration of micro-molecules and macro-molecules and more altered spatial relations in the plasma lemma, tonoplast and membranes of organelles as the cell volume was reduced. Changes in the turgor are directly known to cause changes in growth of many species (Hasio and Acevedo, 1974).

Maintenance of higher internal water status at low soil moisture is an important survival mechanism in any plant species. Drought tolerant cultivars regulate the stomatal function to have some carbon fixation with moisture stress conditions and improve water use efficiency (Yordanov *et al.*, 2003). The maintenance of higher water status with higher stomatal conductance observed in many plants usually occurs together with their deep rooting ability which allows extraction of water from deep soil layers. For example, Ponderosa pine (*Pinus ponderosa*) tree maintained a constant evapotranspiration rate even during dry periods due to deep rooting (Chaves, 1991; Williams *et al.*, 2001). According to TRI Annual report (1980), some drought tolerant tea cultivars exhibited deeper roots (DN=117 cm, CY9=140 cm, TRI 2025=135 cm) than known susceptible cultivars (TRI 2026 =81 cm, TRI 2023 =90 cm, TRI 2024= 110 cm).

The changes in mean photosynthesis rate, stomatal conductance and rate of transpiration of different cultivars are presented in Fig. 2 (a), (b) and (c), respectively.

Drought tolerant ability of tea

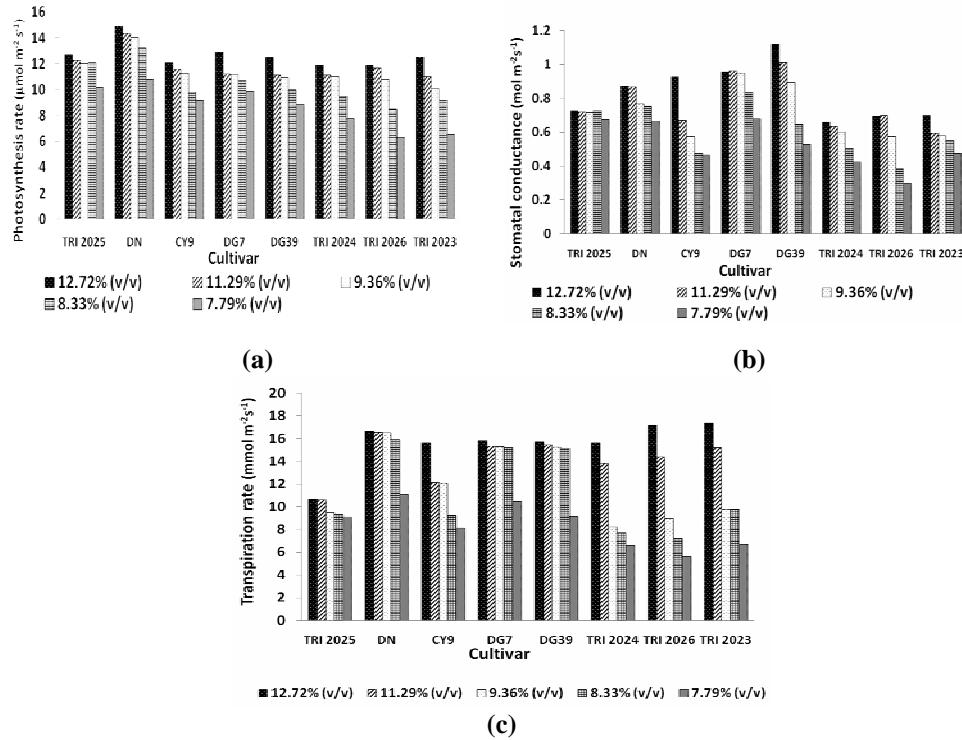


Fig. 2. Change in (a) rate of photosynthesis, (b) stomatal conductance and (c) rate of transpiration with gradual depletion of soil moisture in different cultivars.

The mean photosynthesis rates diminished with gradual decrease in soil moisture content and were significantly ($P < 0.05$) different amongst the cultivars and amongst different moisture levels. The cultivar and moisture level interaction was also significant ($P < 0.05$).

The mean stomatal conductance was significantly ($P < 0.05$) different amongst cultivars and amongst different soil moisture levels ($P < 0.05$) (Fig. 2b). The cultivar and moisture level interaction was also significant ($P < 0.05$). The mean transpiration rate was significantly ($P < 0.05$) different amongst cultivars and different soil moisture levels ($P < 0.05$). The cultivar and moisture level interaction was also significant ($P < 0.05$). At the lowest moisture levels, DN showed the highest ($P < 0.05$) transpiration rate followed by DG7, DG39, TRI 2025, CY9 and was lowest in cultivars TRI 2026, TRI 2024 and TRI 2023 (Fig. 2c).

With the depletion of LWP and RWC, photosynthesis rate also declined rapidly and the degree of reduction also varied among cultivars. Even with the progression of the drought, the resistant cultivars (TRI 2025, CY9, DG7, DG 39 and DN) maintained comparatively higher photosynthesis rates than the susceptible cultivars (TRI 2023, TRI 2024 and TRI 2026, Fig. 2a). Maintenance of high leaf water potential during drought helps to continue appreciable rates of photosynthesis and other metabolic processes, and consequently high instantaneous water use efficiency (Handique and Manivel, 1986).

Reduction of leaf water potential leads to reduction of stomatal conductance which progressively inhibits photosynthesis (Tezara *et al.*, 2002). Decrease of photosynthesis due to water stress is a consequence of both stomatal closure causing increased constraints on

carbon dioxide diffusion and decreased chloroplast activity (Ogren and Oquist, 1985). With the depletion of LWP and RWC, stomatal conductance and transpiration rate also declined and the degree of reduction varied among cultivars. The percentage reduction of transpiration rate during the whole period due to moisture stress was highest in drought susceptible cultivars such as TRI 2023, TRI 2026 and TRI 2024. With the progression of the drought, resistant cultivars maintained higher transpiration rates compared to drought susceptible cultivars.

The changes in total soluble sugar content and total chlorophyll content of the different cultivars are presented in Fig. 3(a) and (b), respectively. The total soluble sugar content was significantly ($P < 0.05$) different amongst cultivars and different soil moisture levels ($P < 0.05$). The cultivar and moisture level interaction was also significant ($P < 0.05$). At the lowest moisture levels, DG7 showed the highest total soluble sugar content followed by DG39, TRI 2025, DN and CY9, TRI 2024 and TRI 2023 and TRI 2026.

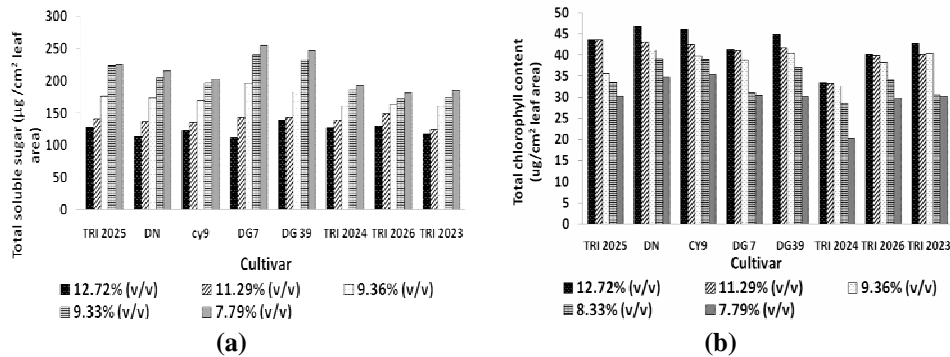


Fig. 3. Change in (a) mean total soluble sugar content and (b) mean total chlorophyll content with gradual depletion of soil moisture in different cultivars.

With the progression of drought, increased total soluble sugar content in leaves could be observed but the degree of this increment with the progression of drought varied between cultivars. Increase of total soluble sugar content with desiccation is a positive character in drought tolerance of a plant. Accumulation of compatible solutes due to stress induced pronounced increase of the activities of enzymes hydrolyzing starch in pigeon pea (Keller and Ludlow, 1993). Increased levels of sucrose contributed towards the maintenance of turgor in coconut (Lakmini *et al.*, 2006). Osmotic adjustments delay stomatal closure during drought. High rates of gas exchange during drought have been reported in plants that adjust osmotically (Abrams *et al.*, 1990). Cultivars DN, DG7, DG 39 and TRI 2025 maintained a comparatively higher water status and higher stomatal conductance, and also high total soluble sugar contents, indicating their ability to withstand drought by osmotic adjustments and probably by extracting water with their deep root systems.

The total chlorophyll contents were not significantly ($P > 0.05$) different amongst cultivars but significantly different amongst moisture levels. The cultivar and moisture level interaction was not significant. However, the total leaf chlorophyll reduced with decreasing LWP and RWC.

Loss of chlorophyll reduces the amount of photons absorbed by leaves and can finally reduce photosynthesis rate and consequent increase in excitation energy, which may lead to the

reduction of oxygen, forming reactive oxygen species (Bosch and Alegre, 2000). Reactive oxygen species damage ATP synthase due to an interaction with increased ion concentration in the chloroplast and slowing the RUBP synthesis (Lawlor and Tezara, 2008). Therefore chlorophyll breakdown can occur during drought for photoprotection of the stressed leaves (Bosch and Penuelas, 2003).

The average stomatal densities and drought susceptibility index of different cultivars are presented in Fig. 4a and b, respectively.

The stomatal densities were significantly ($P < 0.05$) different amongst cultivars. Significantly higher stomatal density was found in cultivar TRI 2023 followed by TRI 2024, TRI 2026, TRI 2025, DG7, DG39, CY9 and DN. Stomatal density also plays an important role when drought tolerance is considered. According to our experimental results, drought tolerant cultivars had lower stomatal densities compared to the known drought susceptible cultivars. De Costa *et al.* (2007) also confirmed greater stomatal densities of the drought susceptible genotypes. However, Chaves (1991) showed that the physiological control of stomatal aperture is more important than the size and frequency of stomata to determine stomatal conductance under water stress.

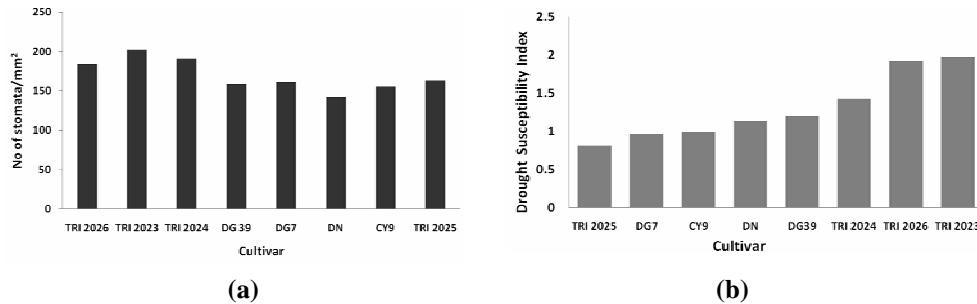


Fig. 4. (a) Stomatal density and (b) drought susceptibility index of different cultivars in the field experiment in Passara area.

Drought susceptibility index was calculated using photosynthetic rate as the reference parameter. TRI 2025 cultivar showed the lowest drought susceptibility index (0.81) ascendingly followed by DG 7, CY9, DN, DG 39, which are also known to be drought tolerant cultivars. TRI 2023 showed the highest drought susceptibility index (1.97) followed by TRI 2026 and TRI 2024 which are known to be drought susceptible.

Cluster analysis was done based on photosynthesis rate, stomatal conductance, transpiration rate, relative water content and total soluble sugar content. Parameters that were significantly different amongst cultivars and significant cultivar \times moisture level interaction were used for the cluster analysis. In this study, drought susceptible and tolerant cultivars could clearly be grouped separately by cluster analysis based on the selected key parameters. The cultivars were grouped into two clear clusters: The drought susceptible cultivars, i.e. TRI 2024, TRI 2026 and TRI 2024 and the known drought tolerant cultivars TRI 2025, DG7, DG39, DN and CY9, indicating that the selected parameters are suitable for cluster analysis in other cultivars as well. This confirms the suitability of this method for analyzing tea cultivars for their drought susceptibility/ tolerance and therefore, the method could be adopted in a tea breeding programme for screening for drought tolerance.

CONCLUSIONS

Several contrasting cultivars in terms of their drought tolerance were used to monitor physiological and biochemical changes in response to drought stress and clear differences could be seen in the responses between the two tolerance classes. Based on the measurements they were grouped as tolerant and susceptible cultivars. TRI 2025, CY9, DG7 and DN were grouped into tolerant category and TRI 2023 TRI 2024 and TRI 2026 into susceptible category. The parameters reflecting drought tolerance which could be incorporated into a drought screening procedure were selected in this study. Photosynthesis rate, stomatal conductance, transpiration rate, relative water content and total soluble sugar content could be successfully used to screen the different tea cultivars for drought tolerance.

ACKNOWLEDGEMENT

Staff of the Agronomy Division of Tea Research Institute who contributed in collecting data are highly appreciated. Authors wish to thank Head and staff of the Plant Breeding Division, OIC and staff of the Passara TRI substation and Ms Ganesha Thotawattage of the Biochemistry Division of Tea Research Institute for their help in numerous ways.

REFERENCES

- Abrams, M.D., Kubiske, M.E. and Steiner, K.C. (1990). Drought adaptations and responses in five genotypes of *Fraxinus Pennsylvanica* Marsh: Photosynthesis, water relations and leaf morphology, *Tree Physiol.* 6,305-315.
- Anonymous (2002). The suitability of different clones for the different regions, TRI Advisory Circular, The Tea Research Institute of Sri Lanka, Talawakelle, 3
- Arnon, D. (1949). Copper enzymes in isolated chloroplasts, Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.* 24, 1- 15.
- Bosch, S.M. and Alegre, L. (2000). Changes in carotenoids, tocopherols and diterpenes during drought and recovery and the biological significance of chlorophyll loss in *Rosmarinus officinalis* plants. *Planta.*, 210, 925-931.
- Bosch, S.M. and Penuelas, J. (2003). Photo- and antioxidative protection during summer leaf senescence in *Pistacia lentiscus* L. grown under mediterranean field conditions. *Annals of Botany*, 92, 385-39.
- Bota, J., Stasyk, O., Flexas, J. and Medrano. H (2004). Effect of water stress on partitioning of ¹⁴C- labeled photosynthates in *Vitis vinifera*, *Funct. Plant Biol.* 31,697-708.
- Chaves, M.M. (1991). Effect of water deficit on carbon accumulation. *Journal of Exp. Botany*, 42 (234),1-16.
- De Costa, W.A.J.M. (2004). *Plant Water Relations: Principles and Applications*, Printing unit, University of Peradeniya, Sri Lanka. Pp. 580.
- De Costa, W.A. J. M, Mohotti, A. J. and Wijeratne, M. A. (2007). Ecophysiology of tea. *Brazilian Journal of Plant Physiol.* 19 (4), 299-332.
- Fischer, R.A. and Maurer, R. (1978). Drought tolerance in spring wheat cultivars, 1. Grain yield responses. *Australian J. of Agric. Res.* 29, 897-912.

- Handique, A.C. and Manivel, L. (1986). Shoot water potential in tea, Two and a Bud, 33 (1 and 2), 39-42.
- Hasio, T.C. and Acevedo, E. (1974). Plant response to water deficits, water use efficiency and drought resistance, *Agric. Meteorology*, 14, 59-84.
- Karunaratne, P.M.A.S., Wijerathne, M.A. and Sangakkara, U.R. (1999). Osmotic adjustment and associated water relations of clonal tea (*Camellia sinensis* L.). *Sabaragamuwa University Journal*. 2(1),77-85.
- Keller, F. and Ludlow, M.M. (1993). Carbohydrate metabolism in drought stressed leaves of Pigeon pea (*Cajans cajan*). *J. of Exp. Botany*, 44(265), 1351-1359.
- Lakmini, W.G.D., Nainayake, N.P.A.D. and De Costa, W.A.J.M. (2006). Biochemical changes of four different coconut (*Cocos nucifera* L.) forms under moisture stress conditions. *J. of Agric. Sci.* 2 (3), 1-7.
- Lawlor, D.W. and Tezara, W. (2008). Causes of decreased photosynthetic rate and metabolic capacity in water-deficient leaf cells: A critical evaluation of mechanisms and integration of processes, *Annals of Botany*, 1-19.
- Mohotti, A.J. and Lawlor, D.W.(2002). Diurnal variation of photosynthesis and photoinhibition in tea: effects of irradiance and nitrogen supply during growth in the field. *J. of Exp. Botany*. 53(367), 313-322.
- Morgan, J.M. (1984). Osmoregulation and water stress in higher plants, *Annual Review of a Plant Physiology, Agricultural Research Center, Australia*. 35, 299-319.
- Nainanayake, A.D. (2004). Impact of drought on coconut (*Cocos nucifera* L.): Screening germplasm for photosynthesis tolerance in the field, PhD thesis, University of Essex, UK.
- Ogren, E, and Oquist, G. (1985). Effects of drought on photosynthesis, chlorophyll fluorescence and photoinhibition susceptibility in intact willow leaves. *Planta*. 166 (3),380-388.
- Tezara, W., Mitchell, V., Driscoll, S.P. and Lawlor, D.W. (2002). Effect of water deficit and its interaction with CO₂ supply on the biochemistry and physiology of photosynthesis in sunflower. *J. of Exp. Botany*, 53(375), 1781-1791.
- TRI Annual Report (1980). Report of the Plant Physiology, Plant Propagation and Breeding Division, The Tea Research Institute of Sri Lanka, Talawakelle, 117.
- Wijerathne, M.A. (1996). Some adaptations of the tea plant to dry environment, *Adapting to climate change: Assessments and issues*, Springer, New York, NY. pp. 174-187.
- Williams, M., Law, B.E., Anthoni, P.M. and Unworth, M.H. (2001). Use of simulation model and ecosystem flux data to examine carbon water interactions in ponderosa pine. *Tree Physiol*. 21(5), 287-298.
- Yordanov, I., Velikova, V. and Tsonew, T. (2003). Plant response to drought and stress tolerance, *Bulgarian J. of Physiol*. 187-206.