30 OCT 2014

FINAL REPORT

Research Grant number: RG/2010/BS/01

Title: Investigating plant responses to changing atmospheric carbon dioxide with special emphasis on anatomical and physiological adaptations of stomata in different ecotypes across a range of altitudes

1. Grant number: RG/2010/BS/01
2. Title of Project: Investigating plant responses to changing atmospheric carbon dioxide with special emphasis on anatomical and physiological adaptations of stomata in different ecotypes across a range of altitudes
3. Principal Investigator: Dr. S.W. Ranwala
4. Co-Investigators: Dr. H.I.U Caldera
   Prof. W.A.J.M. De Costa
5. Institute where Research is being carried out: Department of Plant Sciences, University of Colombo, Tea Research Institute
6. Date of award:08/09/2010
7. Date of completion of the project – 30th April 2014
8. Total allocation of funds – One million eight hundred and eighty seven thousand five hundred and eighty six rupees
9. Total spent – Rs. 1,642,259.30
10. Number of research students employed - one
11. Post graduate degree completed with dates - Doctor of Philosophy from the University of Colombo in November 2012.
12. Number of technical assistants and/or labourers employed and period of service - none
13. Publications/communications arising from the project during the reporting period

Full-length Paper in Peer-Reviewed Conference/Symposium Proceedings

Caldera H.I.U., De Costa, W.A.J.M., Wijeratne, M.A. and Ranwala, S.M.W. (2011). Response of stomatal characters of Sri Lankan tea cultivars to environmental change. Proceedings of the International Conference on the Impact of Climate Change on Agriculture, 20 December, 2011. (Eds.) M. Wijeratne, N.S.B.M. Atapattu, W.W.D.A. Gunawardena, P.W.A. Perera and N.Y.. Hirimuthugoda. pp. 114-123. Faculty of Agriculture, University of Ruhuna, Sri Lanka.

Abstract in Peer-Reviewed Conference/Symposium Proceedings

Caldera, H.I.U., De Costa, W.A.J.M., Woodward, F.I., Lake, J.A., Ranwala, S.M.W.(2012). Stomatal response of *Camellia sinensis* (L.) O. Kuntze to elevated carbon dioxide. Proceedings of the 29th New Phytologist Symposium – Stomata 2012, 2-4 July, 2012, Manchester, United Kingdom. pp. 38.

Caldera, H.I.U. (November 2012). Plant responses to environmental change with special emphasis on anatomical and physiological adaptations of stomata across an altitudinal gradient. PhD dissertation, University of Colombo

Section 2

Executive summary of the project

The sessile nature of plants requires them to continually adapt to variations in their environment and stomata play a pivotal role in supporting this function. The present work focused on the response of stomata to environmental gradients. The objectives included identifying and determining a cultivar based variation in *Camellia sinensis* across an altitudinal range; determining the impact of soil water stress on *C. sinensis*; determining the stomatal, anatomical and physiological responses of *C. sinensis* and *Arabidopsis thaliana* ecotypes to elevated atmospheric [CO_2] and elevated temperature under controlled environmental conditions. The *C. sinensis* cultivars in the field study showed a cultivar based response to altitude in their stomatal numbers and dimensions, though stomatal size was a more conserved trait. While leaf thickness increased with altitude, cultivars sampled at the lowest altitude showed the highest palisade:spongy parenchyma ratio (PSR) while estate cultivars showed the lowest PSR at each altitude. Stomatal characters showed a significant relationship with leaf anatomical characters. In the water stress experiment a significant cultivar x treatment effect was seen for weight and relative water content. The popular TRI 2025 cultivar showed a related drought response irrespective of altitudinal origin. The two estate cultivars used in the study (CY9 and DT-1) also showed a similar drought response.

Many *A. thaliana* ecotypes increased their stomatal density in response to elevated CO_2, but stomatal size was not as plastic in response to both elevated CO_2 and temperature. The stomatal dimensions of *C. sinensis* were responsive to CO_2 but stomatal numbers did not vary with increasing [CO_2]. However, stomatal conductance decreased by 25%, suggesting an aperture level control. Elevated CO_2 increased the net photosynthetic rate and leaf thickness in *C. sinensis*.

Section 3 -Report in detail

1. Introduction/background

The atmospheric carbon dioxide concentration ([CO_2]) is predicted to double from 379ppm within the next 100 years. An average warming of about 0.2^0C per decade is projected in terms of global mean surface temperature. Other climatic changes such as droughts and extreme precipitation events due to changes in atmospheric water vapour levels are also envisaged. [CO_2] and ambient temperatures are key abiotic requirements necessary for plant growth. Plants would respond to these environmental changes by possibly species specific anatomical and physiological alterations which may include reduction in number of stomata, reduction in stomatal conductance, increased leaf thickness and alternations in sink status.

Stomata are small pores present on the aerial parts of plants, predominantly on the abaxial surface (under surface) of leaves. They play a key role in regulating carbon dioxide and water vapour exchanges between plants and their surrounding environment. As such, stomatal physiology influences both the carbon and water balances of vegetation, influencing plant growth and water use. Therefore, elucidation of the short- and long-term modifications/adaptations of stomatal anatomy and physiology in response to increasing atmospheric carbon dioxide is imperative for understanding the response of plants to climate change.

The present study focuses on investigating the fundamental aspects of the response of stomatal physiology to increasing atmospheric [CO_2] and its interaction with other key environmental factors such as soil water deficit and atmospheric vapour pressure deficit. The investigations used two plant species, namely, *Arabidopsis thaliana* (a model plant species with a fully-sequenced genome, with which in-depth investigations on the fundamental aspects can be carried out) and Tea, *Camellia sinensis,* (an economically-important agricultural crop grown across the whole range of altitudes in Sri Lanka).

Arabidopsis thaliana (Brassicaceae) is a small, predominantly selfing annual herb native to temperate regions of the world. Inherent advantages of using it as a test plant include: a short growth cycle, need for limited growth space and a small and completely sequenced genome. Another advantage is the availability of a large number of naturally occurring genotypes and laboratory-induced mutants. Limited research has been carried out on the response of *Arabidopsis* ecotypes from diverse altitudes to environmental changes.

Tea [*Camellia sinensis* (L.) O. Kuntze)] provides the highest export earnings in the Sri Lankan agricultural export sector and the tea industry has considerable socio-economic impacts on the country. The productivity of tea plantations is greatly influenced by climatic factors such as rainfall and temperature. Furthermore, climatic changes that influence alterations in the physiological and anatomical features of the leaf would directly affect the commercially valuable shoot tip. Thus, the predicted changes in the future climate would have far reaching consequences on tea yield and the industry as a whole. Interest in the response of Sri Lankan tea cultivars to climate change is relatively new and limited research has been conducted in this area.

Stomatal characters of Sri Lankan tea cultivars have not been analysed in detail. As a large number of cultivars are grown at different altitudes it is possible that stomatal characters have been selected and respond to the environmental gradients represented by this range of altitudes. The anatomy of the leaf is an important determinant of crop productivity due to its role in carbon assimilation through photosynthesis. However, to date, there have not been any detailed studies on the internal anatomical variation of the tea leaf, both in different cultivars as well as across the different tea growing regions of Sri Lanka. Furthermore, limited research on inter-cultivar variation of stomatal characters and leaf anatomy has meant that relationships between these parameters, which may provide valuable insights into the ecological and physiological basis of plant responses to environmental change, have also received scant attention.

The present research work sought to evaluate plant responses to environmental variations under both controlled environmental and natural conditions. Controlled environmental change experiments were carried out in growth chambers at the Sir David Read Controlled Environment Facility, Department of Animal and Plant Sciences, University of Sheffield, United Kingdom. Field based trials were conducted at the plantations of the Tea Research Institute, Sri Lanka. This included studies across an altitudinal gradient at St. Coombs Estate, Talawakelle (1382m amsl), St.Joachim Estate, Ratnapura (29m amsl) and at the Hantane Tea Estate (762m amsl).

Along with growth parameters, anatomical and physiological differentiation of stomatal characteristics in a range of cultivars were studied under these conditions, as well as *in situ* to determine possible relationships between stomatal characters, environmental conditions and tea yield. This aspect along with studies on impact of drought stress on tea cultivars will broaden the knowledge base of Sri Lankan tea cultivars. In the long term the study may help in providing suitable adaptation measures to enhance tea production under future climate change scenarios.

2. Scientific scope of the project (objectives)

In consideration of the knowledge gaps indentified above in the introduction, the objectives of the study were twofold.

The study on Sri Lankan tea of the research project aimed to:

- Identify cultivar based stomatal, anatomical and physiological differentiation of *Camellia sinensis* across an altitudinal range - Objective 1

- Determine the stomatal, anatomical and physiological response of *Camellia sinensis* cultivars across a natural altitudinal and environmental range to changes in [CO_2] and temperature - Objective 2

- Determine whether soil water availability interacts with the response of stomatal physiology of *Camellia sinensis* - Objective 3

In addition, the Arabidopsis thaliana and chamber based tea studies of the research project aimed to:

- Determine the stomatal, anatomical and physiological responses of 13 different ecotypes of *Arabidopsis thaliana* to elevated atmospheric [CO_2] and elevated temperature under controlled environmental conditions - Objective 4

- Determine the growth and physiological response of seedlings of *Camellia sinensis* to elevated atmospheric [CO_2] and elevated temperature under controlled environmental conditions - Objective 5

3. Materials and methods

3.1 Study species and outline of experiments

Sampling was carried out on mature tea bushes at tea plantations in Ratnapura (29m amsl), Hantane (762m amsl) and Talawakelle (1382m amsl). Twenty (20) tea cultivars/estate selections (Table 3.1) were sampled for the stomatal and anatomical studies.

3.1.1 Site description for field studies

The field studies for *C. sinensis* were carried out in Sri Lanka (7° 0'N, 81° 0'E), sampling one study site from each of the major tea growing regions demarcated according to altitudinal range.

Low altitude study site – Ratnapura (29m amsl)

Sampling was carried out at Field No. 02, St. Joachim Estate, Low country station, Tea Research Institute, Ratnapura (6° 41' N, 80° 40' E) in the south west of Sri Lanka. The long term mean annual rainfall in the region is 3617mm.The minimum and maximum temperatures are 22.4°C and 31.8°C (Anon, 1997-2011).

Mid altitude study site – Hantane (762m amsl)

Sampling was carried out at the Mid country station, Tea Research Institute, Hantane (7° 26 N', 80° 38' E) in central Sri Lanka. The long term mean annual rainfall in the region is 1900mm while the minimum and maximum temperatures are 19.0°C and 28.0°C respectively (Anon, 1997-2011).

High altitude study site – Talawakelle (1382m amsl)

Sampling was carried out at St. Coombs Estate, Tea Research Institute, Talawakelle (6° 55’ N, 80° 40’ E) in central Sri Lanka. The long term mean annual rainfall in the region is 2250mm while the minimum and maximum temperatures are 14.2°C and 23.4°C respectively (Anon, 1997-2011).

Table 3.1 Description of *C. sinensis* cultivars sampled at each location

	Cultivar	Ratnapura (29m amsl)	Hantane (762m amsl)	Talawakelle (1382m amsl)	Origin of cultivars (O.P. – Open pollinated)
1.	TRI 2025	X	X	X	Assam/ Cambod O.P. progeny
2.	TRI 2016	X			
3.	TRI 2026	X			Assam/ Cambod O.P. progeny
4.	TRI 2027	X	X		Assam/ Cambod O.P. progeny
5.	TRI 2043		X	X	Indo-Chinese (Shan Bansang progeny)
6.	TRI 3025	X		X	TRI 2025 O.P.
7.	TRI 3055	X		X	Assam/ Cambod O.P. progeny
8.	TRI 3014	X		X	TRI 2025 O.P.
9.	TRI 3019		X	X	Assam/ Cambod x DT 95
10.	TRI 3016			X	Assam/ Cambod x DT 95
11.	TRI 4042	X		X	TRI 2023 x TRI 2026
12.	TRI 4049	X		X	Assam/ Cambod x CY9
13.	TRI 4053	X		X	Assam/ Cambod x CY9
14.	TRI 4052			X	Assam/ Cambod x CY9
15.	KEN 16/3	X	X		Kenilworth Estate – Mid country wet zone (WM1)
16.	H1/58	X			
17.	CY 9		X	X	Tangakelle Estate - Up country wet zone (WU1)
18.	PK 2		X	X	Park Estate – Up country wet zone (WU3)
19.	DN		X	X	
20.	DT 1			X	Drayton Estate -Up country wet zone (WU1)

3.1.2 Altitude based plant growth studies of *Camellia sinensis*

Under objective two cuttings of *Camellia sinensis* cultivars grown at the high altitudes were transferred to the low altitude (Ratnapura). The same cultivars obtained from mother bushes at a low altitude were transferred to the higher altitude at the TRI, Talawakelle.

Destructive sampling was carried out at the beginning and end of the experimental period to determine plant growth during that period.

The following parameters were measured

- Shoot weight (fresh and dry)
- Root weight (fresh and dry)
- Leaf area

The following allometric relationships were obtained:

- Relative growth rate
- Net assimilation rate
- Specific leaf area
- Leaf weight ratio
- Root to shoot ratio

3.1.3 Water stress experiment

Plants that were maintained in the nursery were potted at the TRI, Ratnapura (cultivars used - TRI 2025, TRI 2026, TRI 3014, TRI 4049, CY9, DT-1). The experiment was thus modified as it was found that Ratnapura cultivars were the ones that mostly suffer from drought. These plants were allowed to rest for 2 weeks and then brought to the Plant House at the Department of Plant Sciences, University of Colombo to initiate the water stress experiment. After one month of acclimation (normal watering), weeding and fertiliser application was carried out prior to initiating the experiment. The plants of each cultivar were randomly divided to two groups and differential watering was carried out (treatments - well watered and limited life sustaining watering).

Relative water content of the leaves was measured periodically according to the method described by Wijeratne (1996). This involved excising mature leaves from each cultivar for each treatment and measuring the fresh weight, turgid weight and the dry weight following oven drying.

At the end of the experiment a destructive sampling was carried out and the following parameters were measured to assess growth.

- Shoot weight – leaf and stem (fresh and dry)
- Root weight (fresh and dry)

3.1.4 *Camellia sinensis* growth conditions in the controlled environmental chambers

The plants were placed in identical controlled environment chambers (SGC2352/FM Sanyo Gallenkamp, UK) at ambient (400 µmol mol^{-1}) (ppm) and elevated (800 ppm) atmospheric CO_2 conditions for the elevated [CO_2] experiment. In the elevated temperature experiment the mean temperature in the ambient cabinet was 21^0C during the 16 hour photoperiod and 18^0C during the dark period. In the elevated temperature cabinet the mean temperature was maintained at 31^0C during the 16 hour photoperiod and at 28^0C during the dark period.

3.1.5 *Arabidopsis thaliana* experiment

The study used 18 different *Arabidopsis thaliana* ecotypes (Table 3.2) from a diverse altitudinal range (50-1260m). The seeds were obtained from the Nottingham Arabidopsis Stock Centre (NASC), UK and grown according to standard protocols.

These accessions of *A. thaliana* were grown from seed to flowering stage in two identical controlled environment chambers (SGC970/C/HQI Sanyo Gallenkamp, UK) under ambient (400ppm) and elevated carbon dioxide (800ppm) conditions for the elevated carbon dioxide experiment. In the elevated temperature experiment the mean temperature in the ambient cabinet was 21^0C during the 16 hour photoperiod and 18^0C during the dark period. In the elevated temperature cabinet the mean temperature was maintained at 28^0C during the 16 hour photoperiod and at 18^0C during the dark period.

Table 3.2 Description of *Arabidopsis thaliana* ecotypes used in the study

	Ecotype	**NASC ID**	**Location of origin**	**Average altitude (m)**	**Climate data**			
					T_{min} ^{0}C	**T_{max} ^{0}C**	**P_{min} (mm)**	**P_{max} (mm)**
1.	Ba –1	N952	Blackmount, UK	550	4.0	14.7	81.0	164.0
2.	Bla-1	N970	Blanes, Spain	50	11.3	27.7	29.0	71
3.	Can-0	N1064	Las Palmas, Canary Islands, Spain	1260	18.3	25.6	1.0	14.0
4.	Col-0	N1092	Columbia, USA	50	-1.2	18.2	31.0	76.0
5.	Cvi-0	N1096	Cape Verde Islands	1200	22.0	27.0	1.0	80.0
6.	Db-1	N1102	Tenne, Germany	450	-0.3	17.6	38.0	68.0
7.	Edi-0	N1122	Edinburgh, UK	150	4.8	15.8	49.0	112.0
8.	Ksk-1	N1634	Keswick, UK	50	2.9	14.3	83.0	161.0
9.	Lan-0	N1304	Lanark, UK	150	2.2	13.8	71.0	127.0
10.	Lc-0	N1306	Loch Ness, UK	50	0.3	12.1	83.0	184.0
11.	Ll-0	N1338	Llagostera, Spain	50	11.3	27.7	29.0	71.0
12.	Mc-0	N1362	Mickles Fell, UK	790	1.1	13.5	72.0	129.0
13.	Mt-0	N1380	Martuba, Libya	150	12.7	25.3	1.0	65.0
14.	Pla-0	N1458	Playa de Aro, Spain	50	NA	NA	NA	NA
15.	Rsch-4	N1494	Rschew/Starize, Russia	150	-9.6	17.0	41	88
16.	Su-0	N1540	Southport, UK	50	2.0	13.6	74.0	130.0
17.	Ts-1	N1552	Tossa de Mar, Spain	50	11.0	18.0	NA	NA
18.	Wil-2	N1596	Wilna, Lithuania	150	0.0	10.0	NA	NA

T_{min} - Minimum mean monthly temperature

T_{max} - Maximum mean monthly temperature

P_{min} - Minimum mean monthly precipitation

P_{max} - Maximum mean monthly precipitation

NA – not available

3.2 General methodology

3.2.1 Determination of stomatal density-related characters

Three leaves per plant were collected from five plants growing under each treatment in the laboratory based studies using *A. thaliana*. In the field based studies using *C. sinensis* cultivars, the above replication was carried out per cultivar at each study site. Stomatal impressions were taken using the method developed at the University of Sheffield.

The following characters were determined:

1. Stomatal density (SD) – Number of stomata per unit area (mm^{-2})
2. Epidermal density (ED) - Number of epidermal cells per unit area(mm^{-2})
3. Stomatal index (SI) = stomatal density/[stomatal density + epidermal cell density] x 100)

Guard cell length was measured using a Quantimet 500 image analysis system (Leica, Milton Keynes, Middlesex, UK) coupled to a light microscope (Leitz Laborlux S, Leitz, Germany).

3.2.2 Leaf anatomical studies

One leaf each was sampled from five randomly-selected tea bushes of each cultivar at each altitude. Leaf strips were cut from the middle portion of the leaf lamina and free hand sections were made.

The thickness of the different tissue layers were measured on a light microscope (Model SE, Nikon, Japan) at x400 magnification using a calibrated eye piece graticule. Two different fields of view of the transverse section of each leaf were observed avoiding the mid rib.

The thickness of the total leaf, palisade and spongy parenchyma layers were measured and these values were used to calculate the following allometric ratios:

1. Palisade layer thickness: spongy parenchyma thickness (PSR)
2. Palisade layer thickness: total leaf thickness
3. Spongy parenchyma thickness: total leaf thickness

3.2.3 Porometry

Porometry was carried out on consecutive days using a porometer (AP4-UK/Delta –T Devices Limited). Using this machine stomatal conductance/ transpiration and light intensity was measured.

3.2.4 Measurement of carbon dioxide

Measurement of carbon dioxide at the three study sites was carried out using an open system Li-6400 infrared gas analyzer (Li-COR, Lincoln, NE, USA) at Ratnapura, Hantane and Talawakelle.

3.2.5 Leaf physiology of *Camellia sinensis* maintained in growth chambers (UK study)

Physiological measurements (photosynthetic rate, stomatal conductance, transpiration rate and inter cellular CO_2 concentration) of the *C. sinensis* plants grown in Sheffield were recorded using an open system Li-6400 infrared gas analyzer (Li-COR, Lincoln, NE, USA). Fully exposed, mature leaves were used for physiological measurements. The measurements were made under each treatment condition (CO_2 concentration of approximately 400ppm or 800ppm, relative humidity 55%) using the 6 cm^2 leaf chamber without an artificial light source within each growth cabinet. The water use efficiency (WUE) was calculated as the ratio between simultaneously-measured photosynthetic rate and transpiration rates.

3.3 Statistical analysis

All analyses were carried out on the Statistical Analysis System (SAS) version 9.0 (SAS Institute., Cary, N.C).

3.3.1 Effect of altitude and cultivar

Significance of the effects of altitude and cultivar on stomatal and anatomical characters was determined by analysis of variance (ANOVA) procedure of the Statistical Analysis System (SAS) version 9.0 (SAS Institute, Cary, NC, USA). As all cultivars were unavailable at each altitude, data were subjected to unbalanced ANOVA, using GLM (General Linear Model) procedure of SAS. Multiple comparisons among means was carried out by the Duncan's Multiple Range Test at $p = 0.05$ level. The standard error of each mean was calculated using the SAS software and is represented by vertical bars on the graphs.

3.3.2 Factor Analysis and Cluster analysis

The leaf anatomy and stomatal data of the tea cultivars were subjected to principal component analysis using ones as prior communality estimates. The principal axis method was used to extract the components, followed by varimax (orthogonal) rotation. In interpreting the rotated factor pattern, an item was said to load on a given component if the factor loading was 0.60 or greater for that component, and was less than 0.60 for the other. Then average linkage cluster analysis was carried out using principal component scores in order to cluster the similar tea cultivars/ *A. thaliana* ecotypes.

4. Results

4.1 Identifying a cultivar based differentiation across an altitudinal gradient

4.1.1 Stomatal density related characters

The stomatal characters showed significant variation ($p<0.0001$) based on the altitude from which the cultivars were sampled. The plants growing at the lowest altitude showed a significantly higher SD and SI followed by the high and mid altitude plants (Figs.4.1a & c). The ED was similar at the highest and the lowest altitudes (Fig.4.1b).

The direction of change of SD and SI in response to the altitude and its magnitude differed significantly for the different tea cultivars. For instance, TRI 3014 showed a decrease in SD with increasing altitude while TRI 3025 showed an increase. Conversely, TRI 2043 did not show a significant variation in SD or SI. In terms of ED, CY9 showed an increase with increasing altitude while KEN 16/3 showed a decrease. Thus, the altitude x cultivar interaction for the SD, ED and SI was highly significant ($p<0.0001$) indicating that different cultivars responded differently to the environmental change represented by the altitudinal gradient.

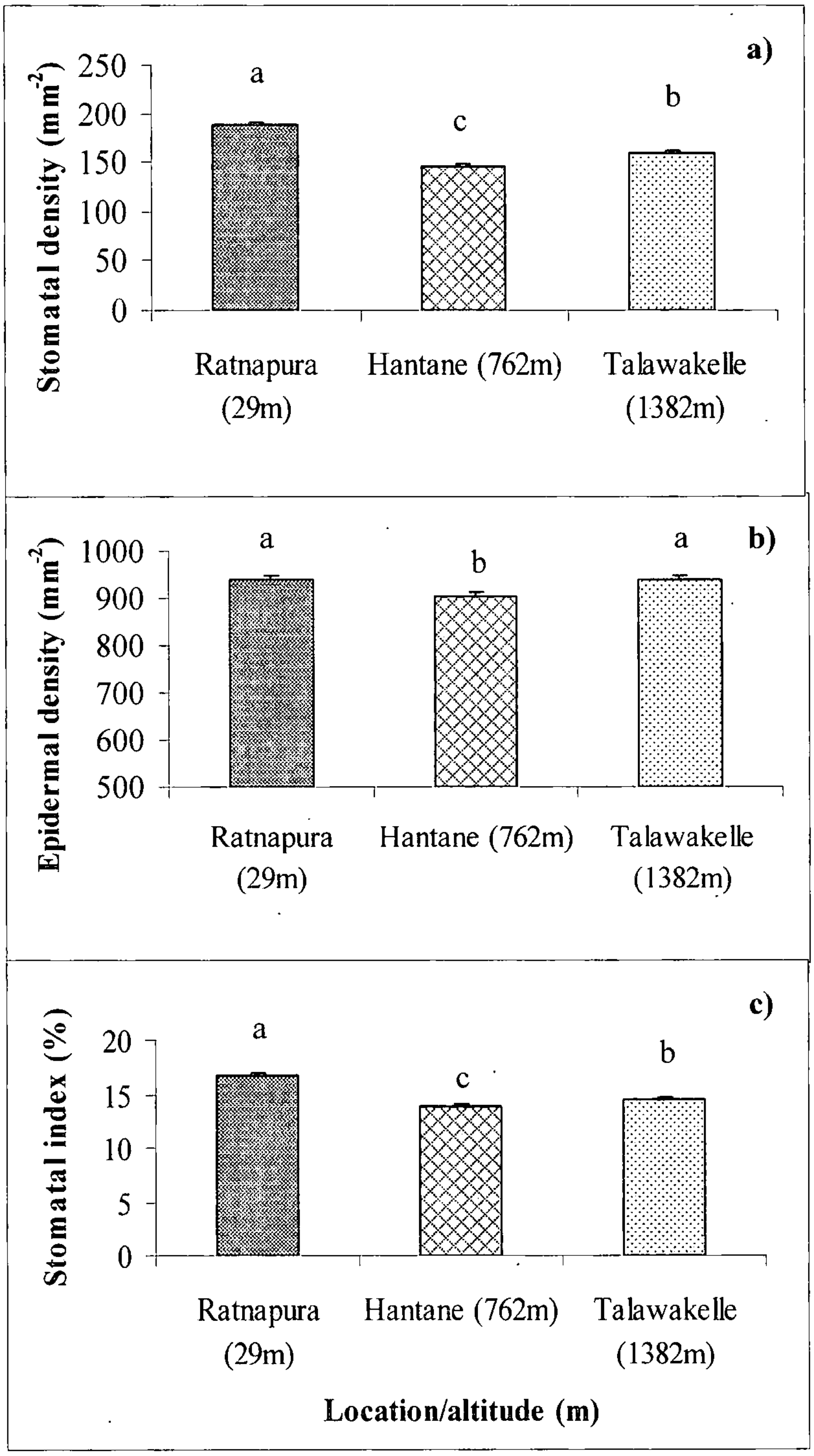


Fig. 4.1 Variation of stomatal density (a) epidermal density (b) and stomatal index (c) of *C. sinensis* cultivars grown across an altitudinal gradient; Error bars = SE mean. Number of samples were 600, 1125 and 900 for Hantane, Talawakelle and Ratnapura respectively. Letters indicate significant differences among responses to location according to Duncan's multiple range test ($p<0.05$).

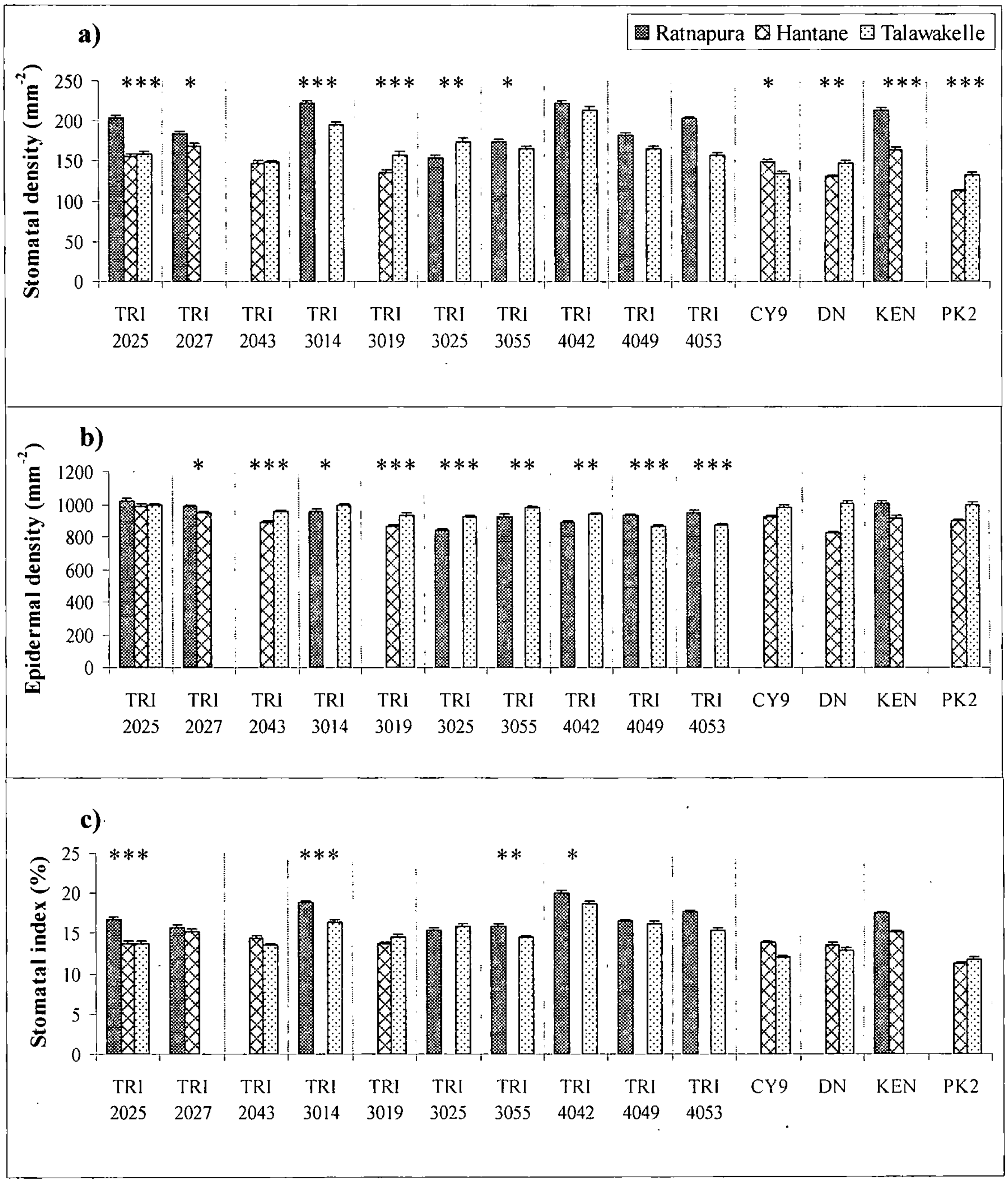


Fig. 4.2 Variation in stomatal density (a) epidermal density (b) and stomatal index (c) of *Camellia sinensis* cultivars across an altitudinal gradient. Error bars = SE mean. *n*= 75. (Significant at*$p<0.05$; **$p< 0.001$; ***$p<0.0001$)

However, frequency distributions in the percentage change in SD and SI with increasing altitude showed that a majority of the cultivars (i.e. a greater frequency) showed reductions in SD and SI in response to increasing altitude (Figs.4.3a & b).

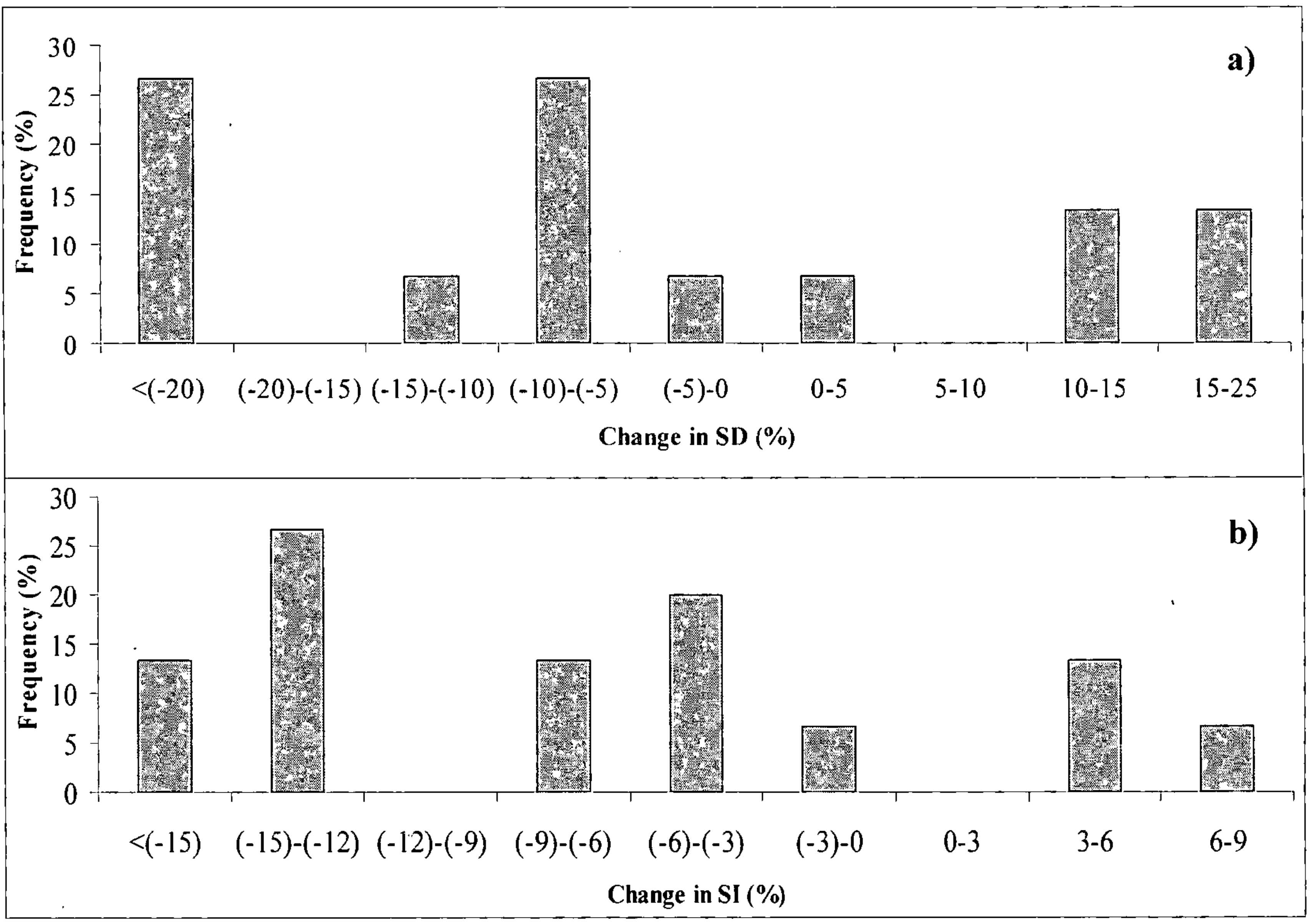


Fig. 4.3. Frequency distribution of the percentage change in stomatal density (a) and stomatal index (b) with increasing altitude. Frequency is the % of tested tea cultivars showing a given % change in a stomatal character in response to increasing altitude.

4.1.2 Leaf anatomy of selected Sri Lankan tea cultivars

All cultivars had a uniseriate epidermis and the spongy parenchyma occupied a large portion of the leaf transverse section in all cultivars at all altitudes. The thickness of the leaves increased with altitude, with plants growing at the highest altitude having the thickest leaves and also the thickest palisade and spongy parenchyma layer (Figs. 4.4a, b & c). The cultivars at the lowest altitude had the thinnest leaves and spongy parenchyma layer. There was a highly significant ($p<0.0001$) variation amongst the cultivars for the variables of interest and a significant cultivar x altitude interaction was also seen.

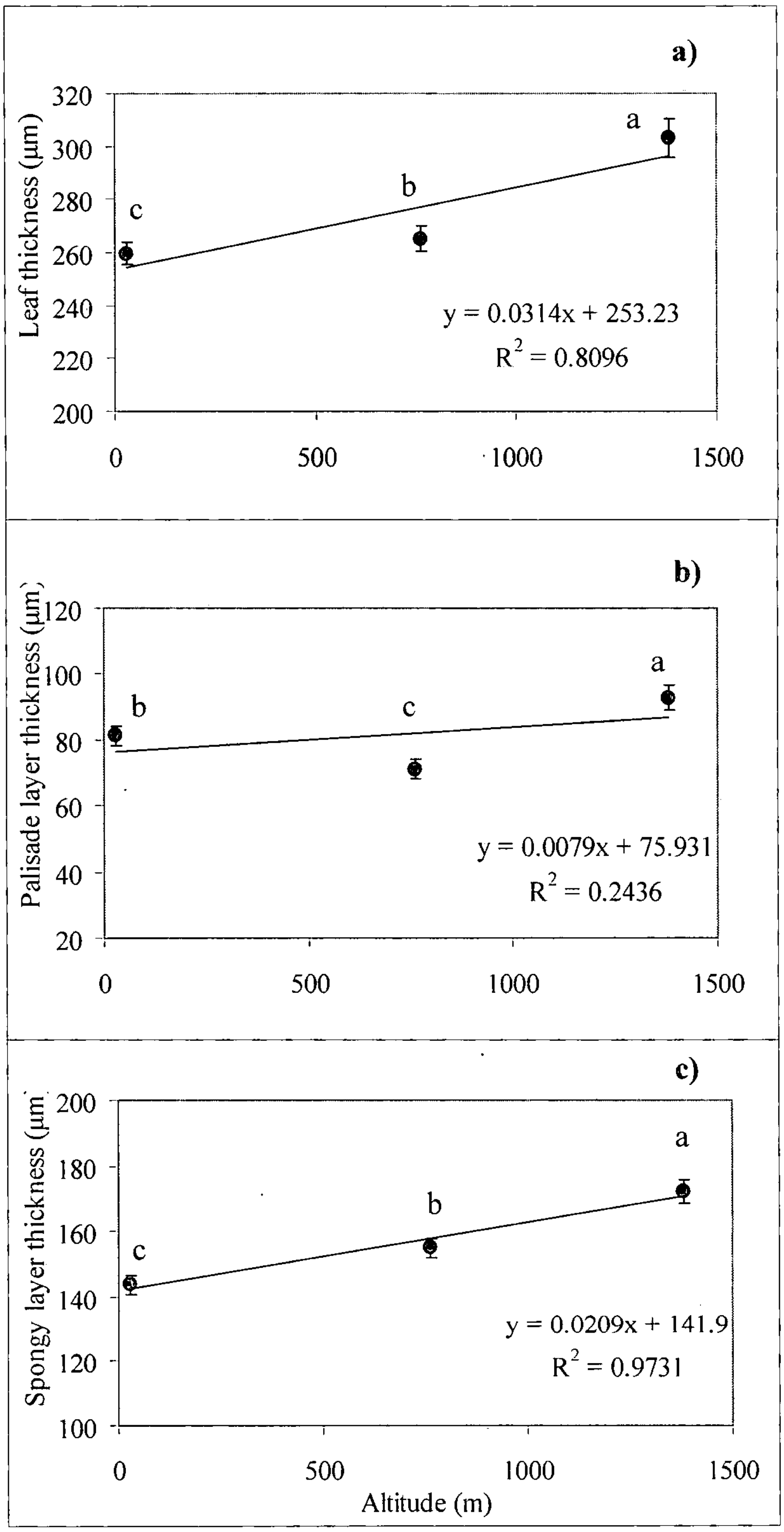


Fig. 4.4 Variation of leaf (a) palisade layer (b) and spongy parenchyma layer (c) thickness of *C. sinensis* cultivars across an altitudinal gradient; Error bars = SE mean. Regressions are from means for each altitude based on 120 (low), 80 (mid) and 150 (high) data points. Letters indicate significant differences among responses to altitude according to Duncan's multiple range test ($p<0.05$).

A significant cultivar x altitude interaction was seen for the allometric ratio of spongy: total leaf thickness, PSR and palisade: total leaf thickness ratios of tea cultivars. This showed that there was a cultivar specific response to altitude for the calculated anatomical ratios.

The spongy parenchyma to leaf thickness ratio separated out under Duncan's Multiple Range Test (DMRT) according to the location as Ratnapura< Talawakelle <Hantane at $p<0.05$. Thus, the cultivars growing at Hantane had the highest ratio of spongy parenchyma to leaf thickness. The same mean separation procedure showed that the cultivars at Hantane had a significantly ($p<0.05$) lower PSR and palisade to leaf ratios in comparison to the other two elevations. The highest PSR was obtained for the cultivars growing at Ratnapura followed by Talawakelle and Hantane (significant at $p<0.05$).

The cultivar based variation showed an interesting difference between the estate cultivars (cultivars developed from selections made from old seedling tea populations existing in various estates) and the improved cultivars introduced by the TRI (Figs. 4.5 and 4.6). At each altitude the estate cultivars had a lower amount of palisade parenchyma when compared to the cultivars introduced by the TRI. For example, the cultivar DT-1 (an upcountry estate cultivar) which had the thickest leaves (323.50 μm) from all cultivars sampled at the three altitudes had one of the lowest PSR and palisade to total leaf thickness ratios. In fact, all the estate cultivars sampled in this study had a thicker spongy layer when compared to the TRI cultivars.

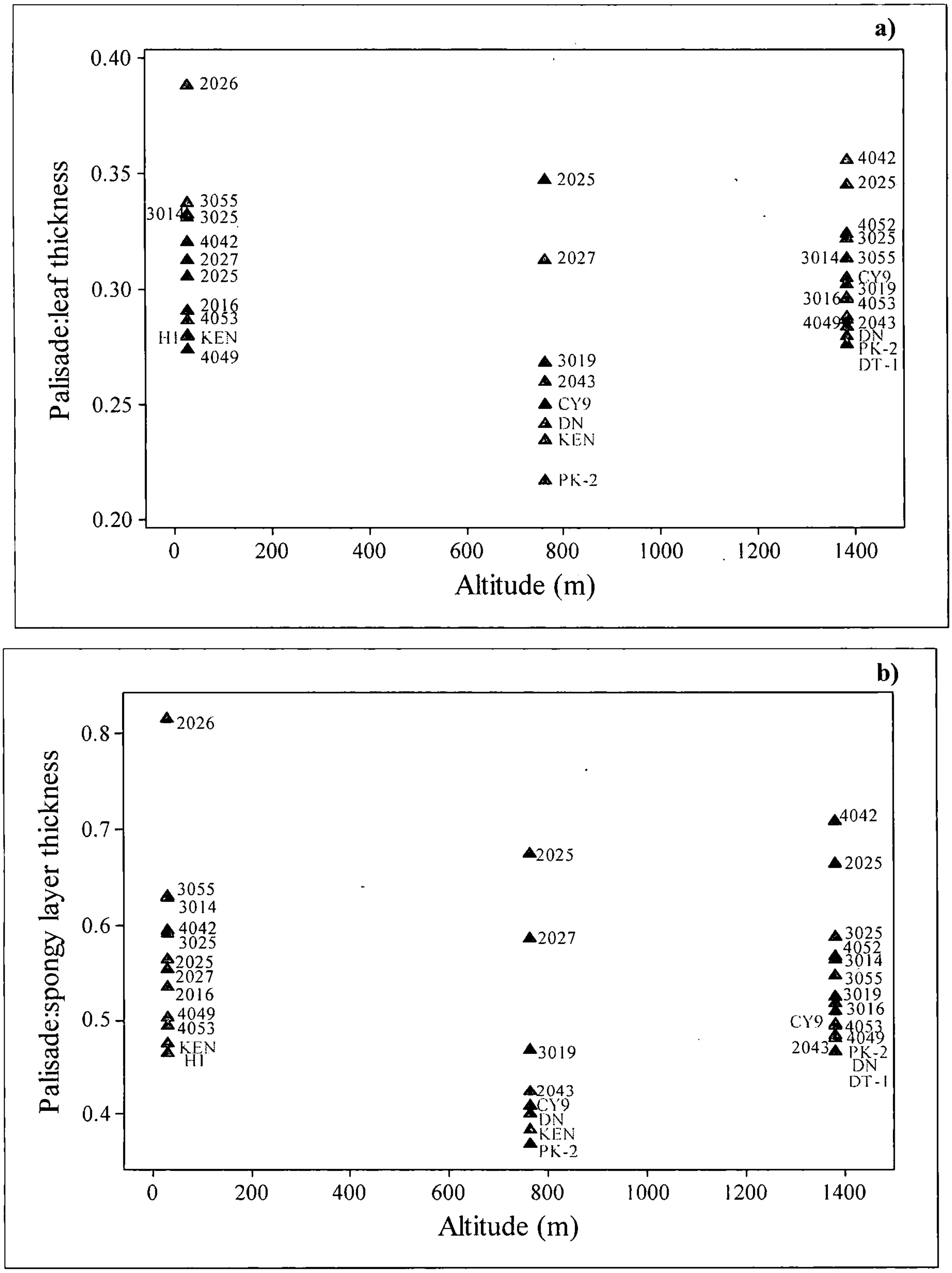


Fig. 4.5 Variation of (a) palisade: leaf and (b) palisade: spongy layer thickness of *C. sinensis* cultivars across an altitudinal gradient. Each data point is a mean of ten replicate measurements. The estate cultivars are shown in red coloured font.

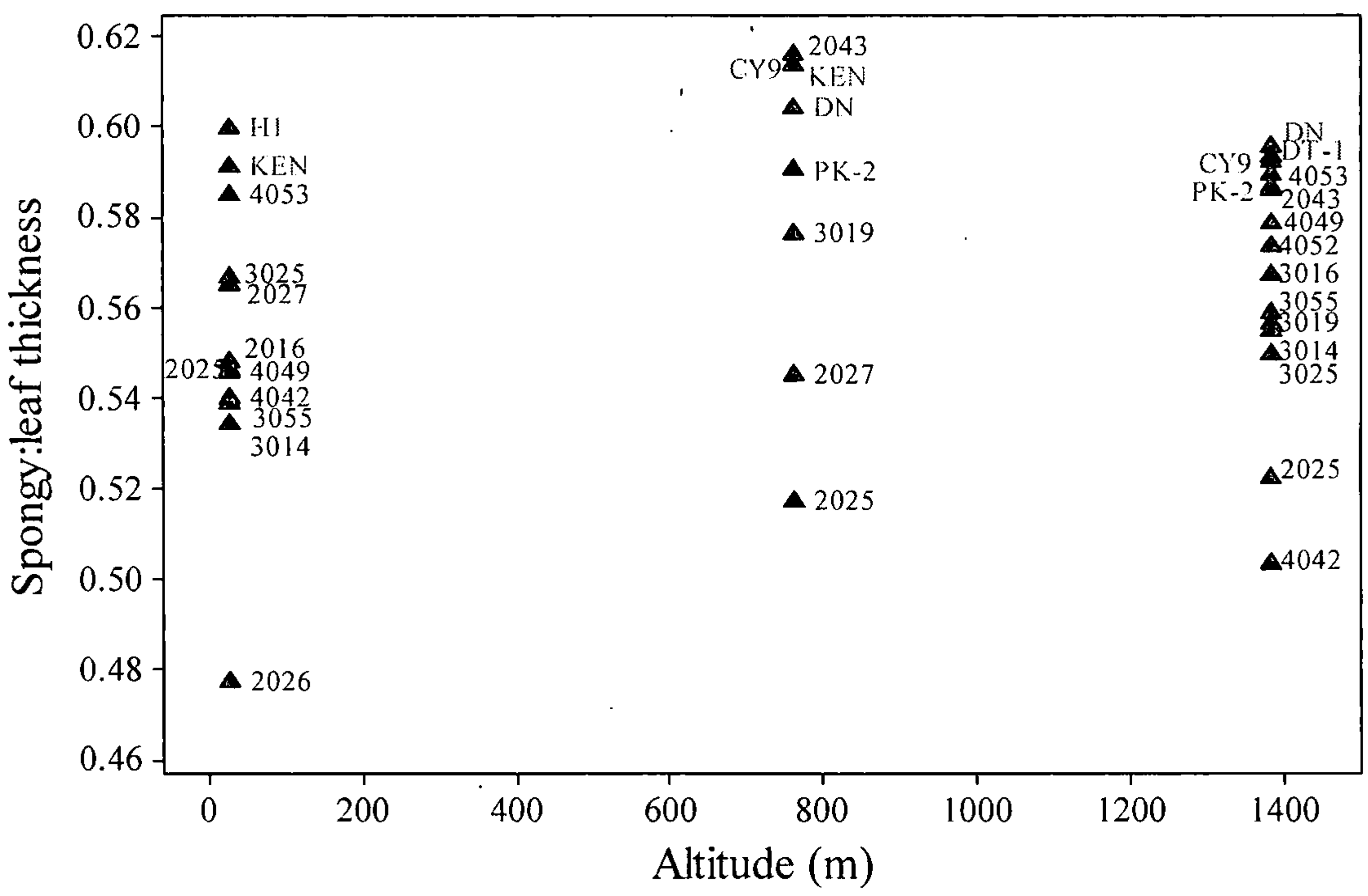


Fig. 4.6 Variation of spongy: leaf thickness of *C. sinensis* cultivars across an altitudinal gradient. Each data point is a mean of ten replicate measurements. The estate cultivars are shown in red coloured font.

4.1.3 Photosynthetic rate

Photosynthetic measurements could not be carried out due to the unavailability of a photosynthetic meter. As advised by a National Science Foundation review board, mean leaf photosynthesis values were obtained from literature. However, as a majority of the cultivars under the present study have not been extensively studied, values could be obtained according to an altitudinal gradient only for one of the most commonly used tea cultivars, i.e. TRI 2025. This value was compared with the anatomical proxy used for photosynthesis in the present study – the palisade to spongy layer ratio (PSR). Kottawa was taken as it is at a comparable elevation to Ratnapura (29m a.m.s.l). Both the PSR value and photosynthetic rate increased with increasing elevation according to the literature cited.

Table 4.1 Comparison of PSR with mean leaf photosynthetic rate for the cultivar TRI 2025

Location	Elevation (m a.m.s.l.)	Mean Leaf photosynthetic rate (μmolm^{-2} s^{-1})	PSR
Kottawa	30	9.1	0.564
Talawakelle	1382	9.3	0.665

(Source: Balasuriya, 2000; De Costa *et al.*, 2007a; Wijeratne *et al.*, 2008)

Duncan mean separation showed that there was a significant difference in the carbon dioxide concentrations at the three study sites (Table 4.2).

Table 4.2 Comparison of CO_2 concentration at the study sites.

Location	Elevation (m a.m.s.l.)	CO_2 concentraion (ppm)
Ratnapura	30	381
Hantane	762	376
Talawakelle	1382	371

4.1.4 Principal Component Analysis and Cluster Analysis to determine the overall response of stomatal and anatomical characters

Only the first three principal components displayed eigen values greater than one which were further confirmed by the results of the scree test. These three components were retained for rotation and accounted for 93% of the total variance. In the analysis the variable palisade layer thickness was scratched out as it loaded almost equally onto more than one component.

The measured variables and the corresponding factor loadings showed that three items loaded to the first component which consisted of leaf anatomy allometric ratios. Stomatal density related characters loaded to the second component and included the SD, SI and PCI (Fig.4.7). Two items loaded to the third component and these were the leaf and spongy parenchyma thickness. The fourth factor had three items and these were the ED along with the GCL and aperture length.

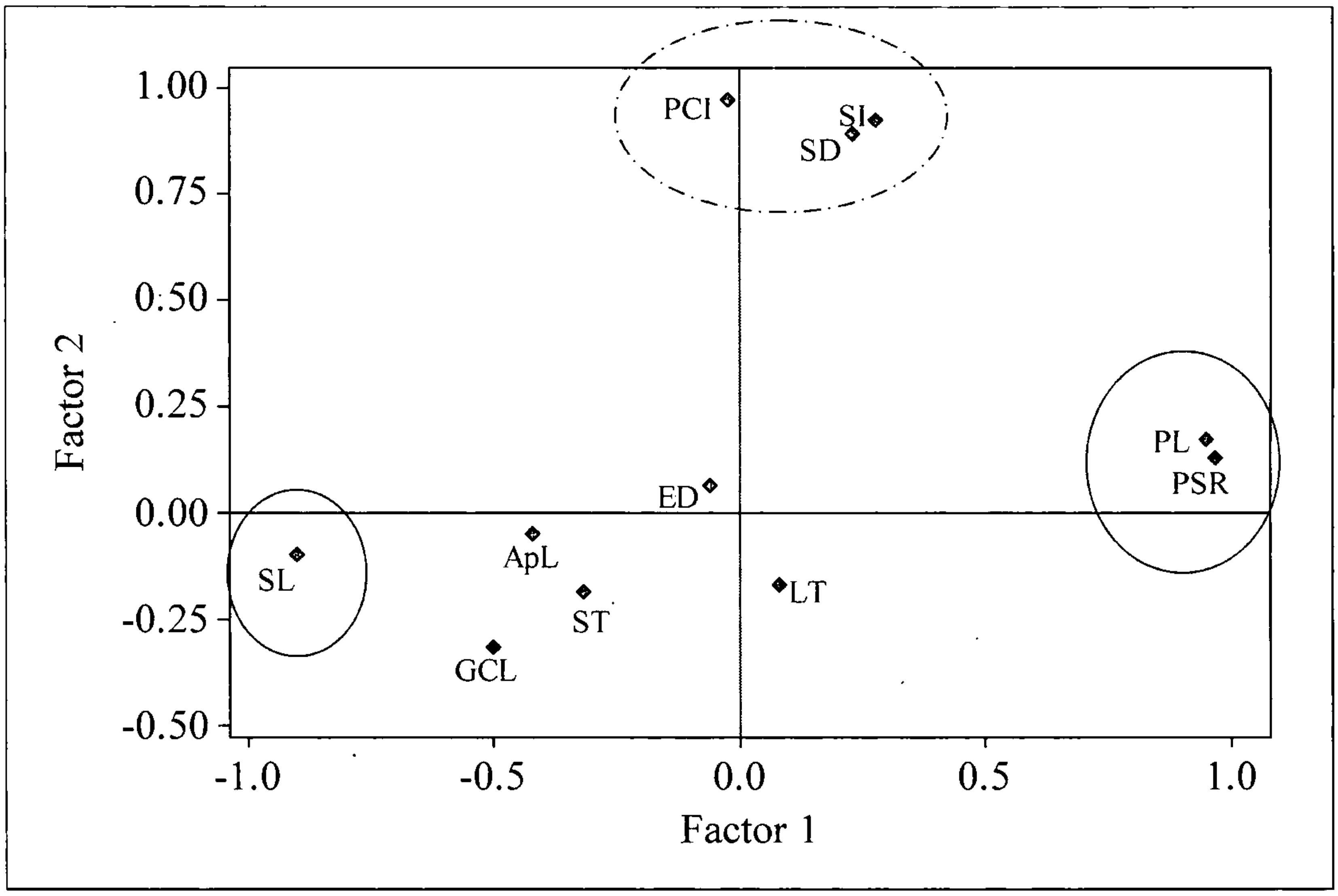


Fig. 4.7 Factor analysis for stomatal characters and leaf anatomical data of *C. sinensis* cultivars across an altitudinal gradient. Solid circles surround traits that loaded on to factor 1 while those circled with dashed lines indicate those that loaded to factor 2. SD=stomatal density; ED=epidermal density; SI=stomatal index; GCL=guard cell length; ApL=aperture length; PCI=potential conductance index; LT= leaf thickness; ST=spongy mesophyll thickness; PSR=palisade to spongy mesophyll ratio; SL=spongy layer to total leaf thickness ratio; PL=palisade to total leaf thickness ratio.

The dendrogram (Fig. 4.8) obtained from average linkage cluster analysis showed seven clusters which diverged from around 0.85 Euclidean distance (Table 4.3). MANOVA analysis of the clusters showed them to be significantly different from each other (Wilks' Δ=0.00871, *F* (24, 88.4) = 10.66, p<0.0001).

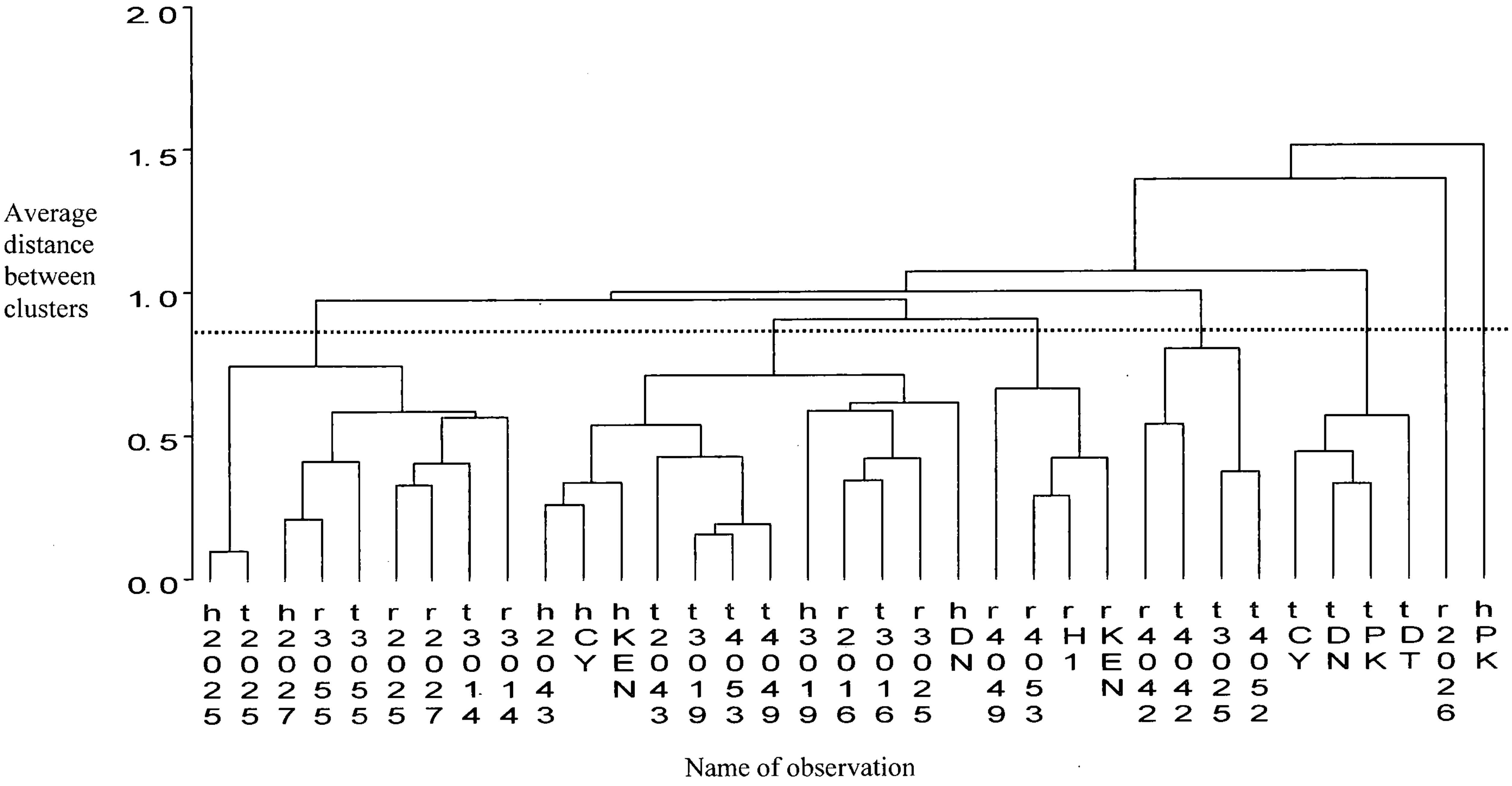


Fig. 4.8. Dendrogram obtained by average linkage cluster analysis of leaf anatomy and stomatal data of *C. sinensis* cultivars across an altitudinal gradient. The prefixes before the cultivar name denote; r= cultivars sampled from Ratnapura, h= cultivars sampled from Hantane, t= cultivars sampled from Talawakelle.

Table 4.3 The composition of clusters derived from the dendrogram

Cluster number	Cultivars within the cluster
Cluster 1	h2025, t2025, h2027, r3055, t3055, r2025, r2027, t3014, r3014
Cluster 2	h2043, hCY9, hKEN, t2043, t3019, t4053, t4049, h3019, r2016, t3016, r3025, hDN
Cluster 3	r4049, r4053,rH1, rKEN
Cluster 4	r4042, t4042, t3025, t4052
Cluster 5	tCY9, tDN, tPK-2, tDT
Cluster 6	r2026
Cluster 7	hPK-2

The prefixes before the cultivar name denote; r= cultivars sampled from Ratnapura, h= cultivars sampled from Hantane, t= cultivars sampled from Talawakelle

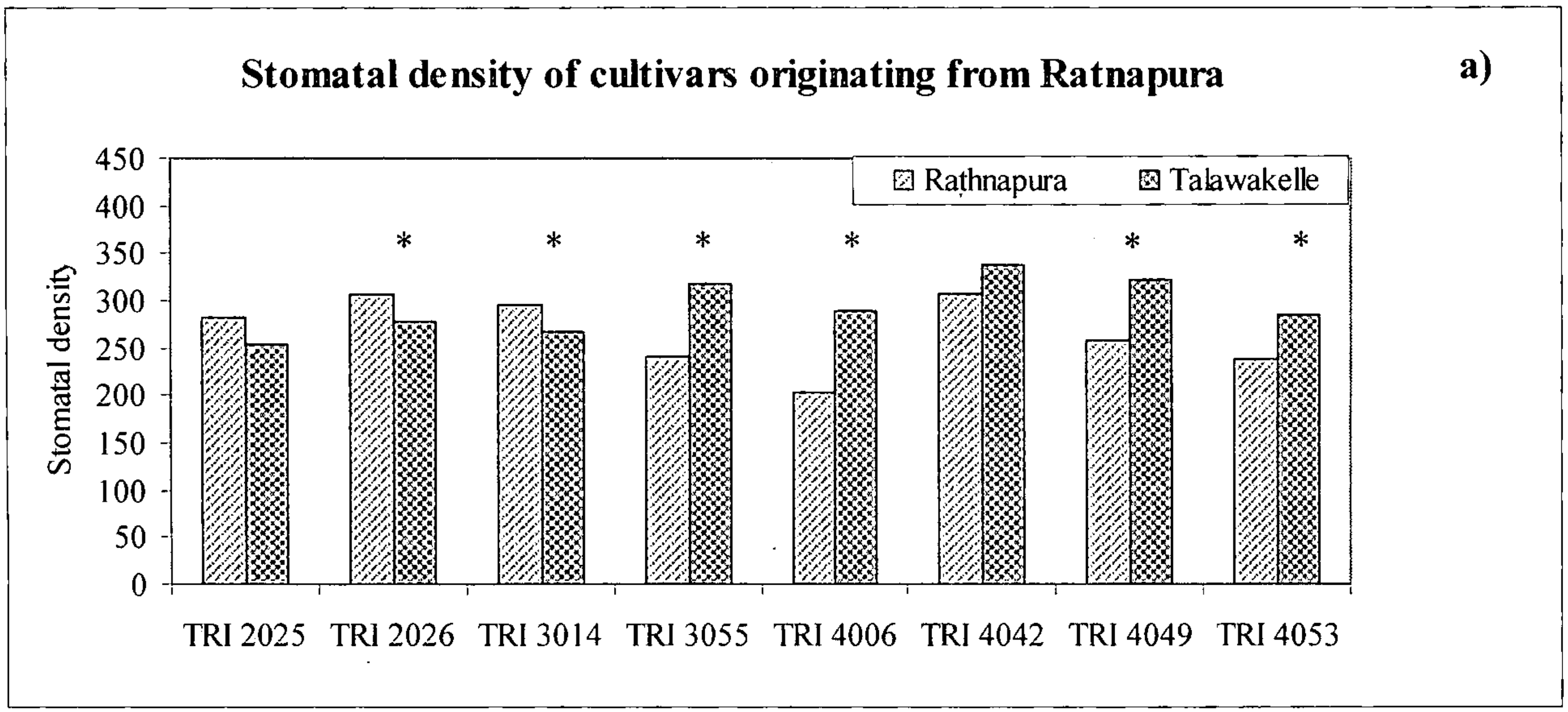
4.2 Elevation experiment

4.2.1. Stomatal characteristics

Analysis was carried out separately for the cultivars that were propagated from mother plants from Ratnapura and Talawakelle (origin) in order to see the effect of altitude when they were grown at the two locations (high and low altitude).

The Ratanapura origin plants had a significantly ($p<0.001$) higher SD and SI than the Talawakelle origin plants. Some of these cultivars such as TRI 2026, TRI 3014 showed a significantly higher SD while TRI 2025, TRI 2026, TRI 3014 and TRI 4049 showed a significantly higher SI when the plants were grown at Ratnapura (i.e the lower altitude) (Fig 4.9 a and b).

TRI 2025, TRI 3019 out of the Talawakelle origin plants had a higher SD when grown at the lower altitude (Fig. 4.10 a and b). TRI 2025 and DN showed a higher SI at the lower altitude.



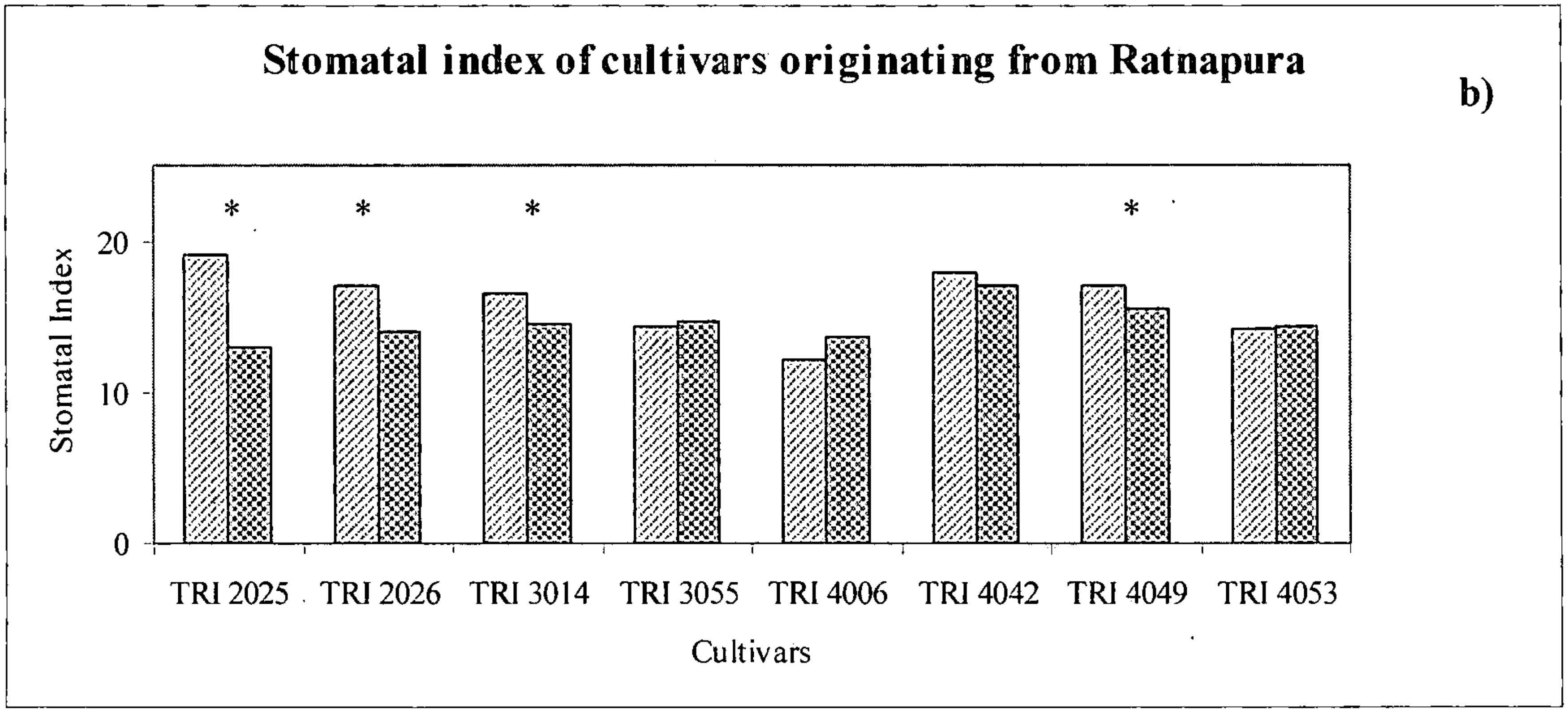


Fig. 4.9 Variation in stomatal density (a) and stomatal index (b) of *Camellia sinensis* cultivars originating from the low altitude and grown at high and low altitudes (Significant at$*p<0.05$)

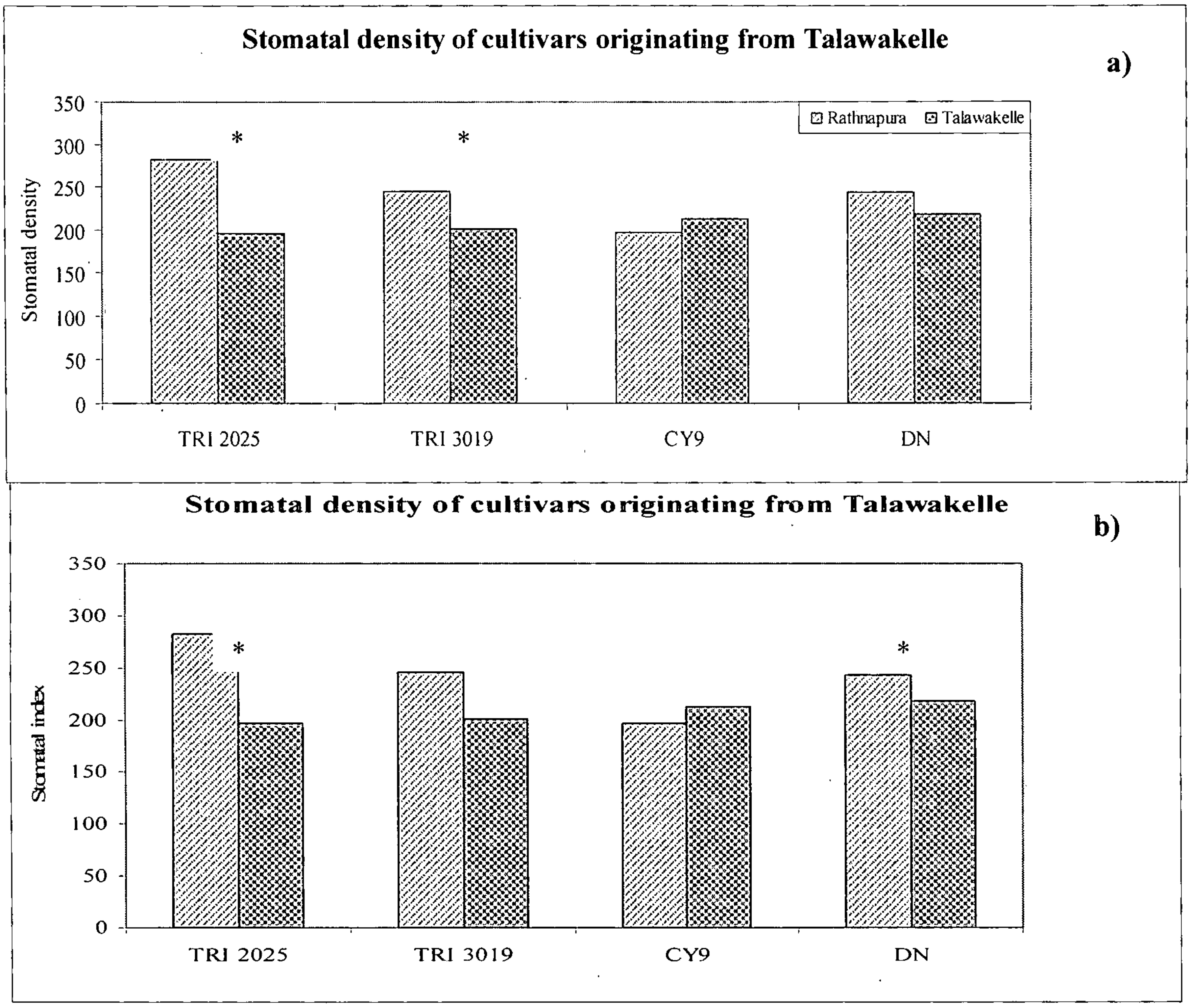


Fig. 4.10 Variation in stomatal density (a) and stomatal index (b) of *Camellia sinensis* cultivars originating from the high altitude and grown at high and low altitudes (Significant at*$p<0.05$)

4.2.2. Stomatal conductance of tea cultivars

A cultivar effect was not observed for stomatal conductance in the cultivars propagated from Talawakelle mother plants and grown at the same high altitude. However, a significant ($p<0.001$) cultivar effect was observed in Talawakelle for the plants which originated from mother bushes in Ratnapura.

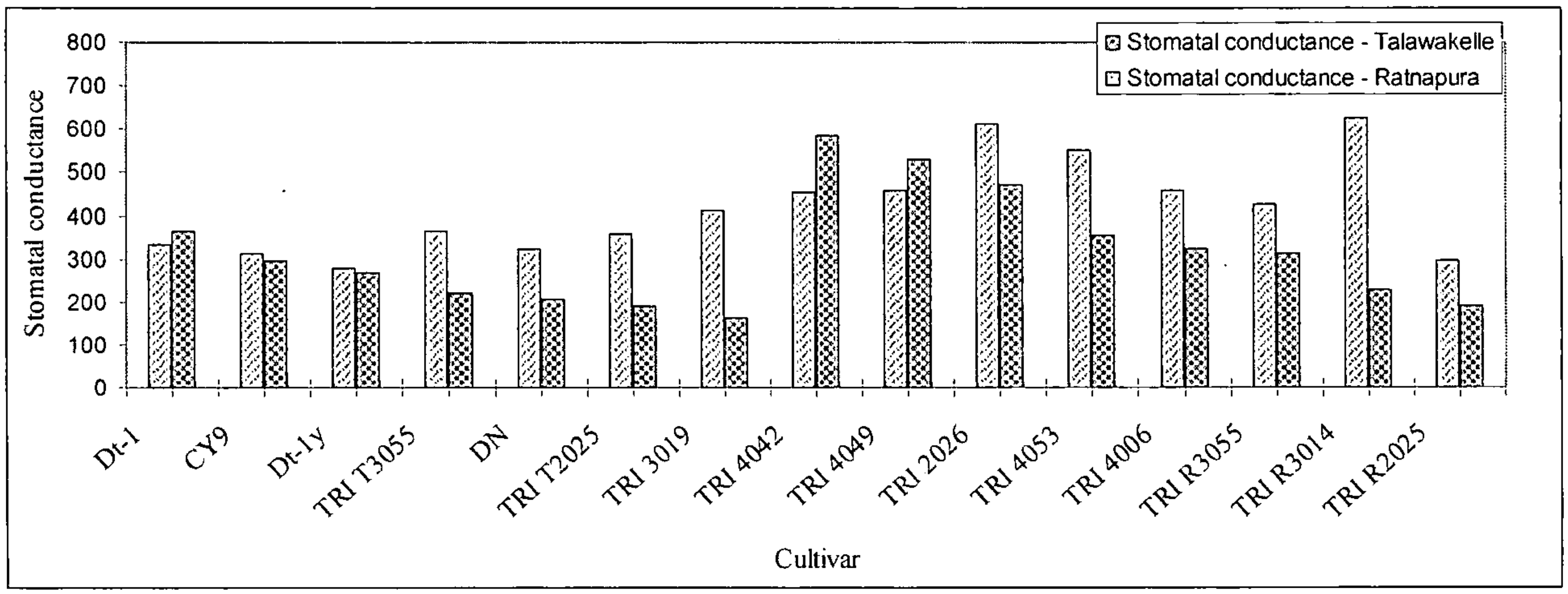


Fig. 4.11 Variation in stomatal conductance (mmolm^{-2}s^{-1}) of *Camellia sinensis* cultivars originating from the high and low altitudes measured at two altitudes (The prefixes before the cultivar name denote; R= cultivars originating from Ratnapura and T = cultivars originating from Talawakelle) (Significant at*p<0.05)

When stomatal conductance was measured in the plants growing at Ratnapura it was seen that cultivars propagated from Talawakelle mother plants showed no cultivar effect. The cultivars propagated from Ratnapura mother plants however showed a significant cultivar effect when grown at the same low altitude. Thus, irrespective of where they were grown the cultivars originating from the lower altitude showed a cultivar based difference in its stomatal conductance.

When comparing the stomatal conductance values it was also seen that irrespective of where the plants were propagated from, cultivars growing at Ratnapura, i.e. the lower elevation had a significantly ($p<0.001$) higher stomatal conductance. Furthermore, the cultivars propagated from the lower altitude had a significantly higher stomatal conductance than those propagated from the higher altitude (Table 4.4).

Table 4.4 Stomatal conductance of cultivars sampled at the two altitudes

Origin of mother plants	Stomatal conductance ($mmolm^{-2}s^{-1}$)	
	Ratnapura	Talawakelle
Low elevation (29m)	484.25 aA	373.68 aB
High elevation (1382m)	335.86 bA	241.06 bB

a-b values in columns within a location compare the variation between different altitudes of origin. A-B values compare the response of cultivars originating at different altitudes at one location. Respective means connected by the same upper-case or lower-case letters are not significantly different at p=0.05 according to the Duncan's Multiple Range Test.

4.2.3 Plant growth studies

Relative growth rate (RGR)

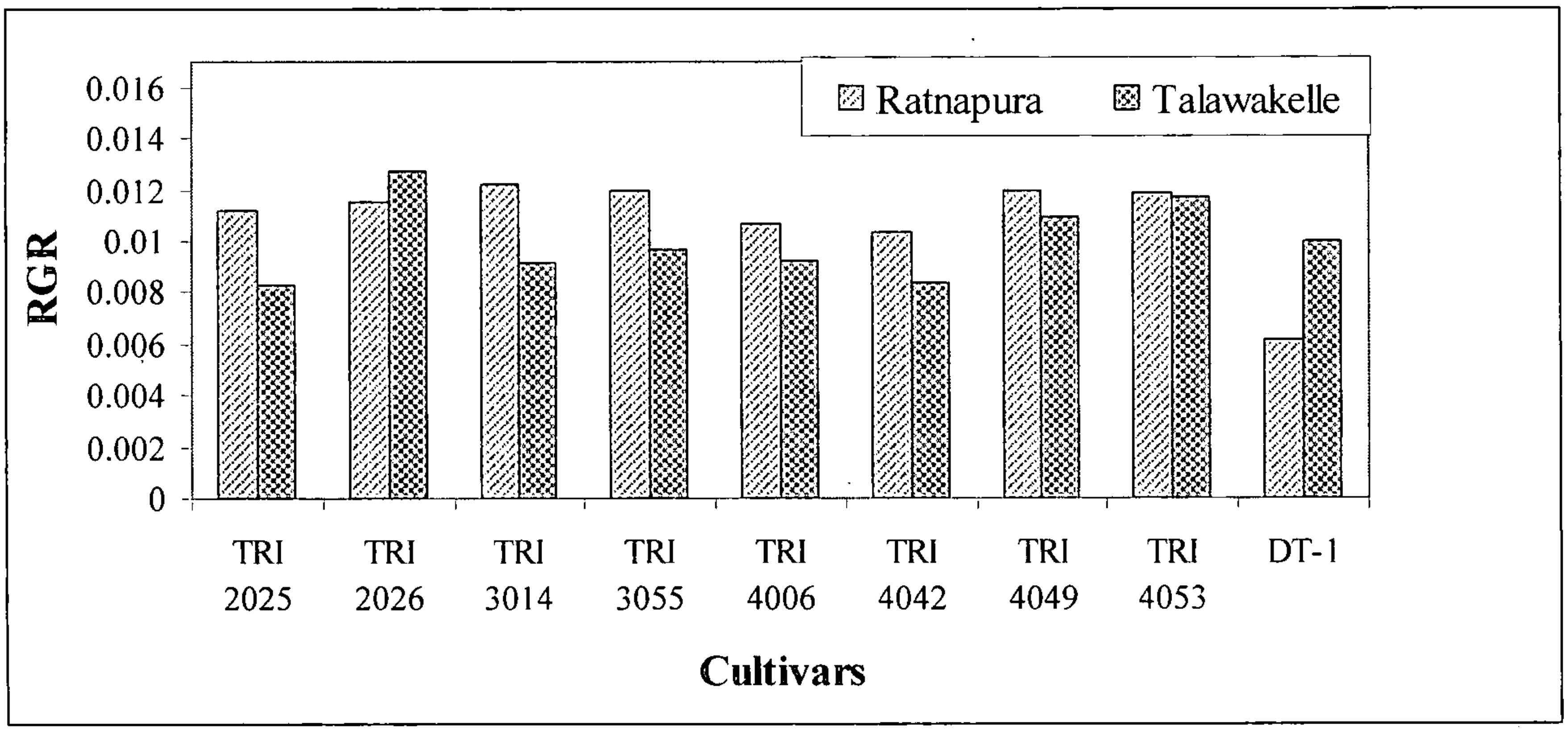


Fig. 4.12 Variation in RGR of *Camellia sinensis* cultivars grown at two altitudes (Ratnapura; 29m and Talawakelle 1382m)

Cultivars originating from Ratnapura showed a significantly higher RGR when grown at the same low altitude site rather than at the higher altitude. DT-1, a high altitude cultivar, showed a lower RGR in Ratnapura and a higher one in Talawakelle.

Leaf area

Cultivars originating from the lower altitude (29m) showed a higher leaf area than those from the higher altitude (1382m) for both the initial and final sampling. However, it was observed that plants grown at the higher altitude for the elevation experiment showed a higher leaf area for both initial and final sampling (Table 4.5).

Table 4.5 Leaf area according to origin of mother plants used to propagate the plants and according to location at which the plants were grown

Origin of mother plants	Leaf area (m^2)	
	Initial	Initial
Low elevation (29m)	0.0179a	0.0179a
High elevation (1382m)	0.0102b	0.0102b
Location of plants		
Low elevation (29m)	0.0156B	0.0557B
High elevation (1382m)	0.0185A	0.0757A

Letters in columns within a sampling compare the variation between different altitudes. Letters indicate significant differences among responses according to origin of mother plants or location according to Duncan's multiple range test ($p<0.05$)

Net Assimilation rate (NAR)

The cultivars grown at the low altitude had a significantly higher NAR. The origin of the cultivars or the cultivars themselves however, did not affect the NAR.

Table 4.6 Net Assimilation Rate according to location at which the plants were grown

Location of plants	Net Assimilation Rate ($gm^{-2}d^{-1}$)
Low elevation (29m)	2.697a
High elevation (1382m)	2.072b

Letters indicate significant differences among responses to location according to Duncan's multiple range test ($p<0.05$)

Specific leaf area (SLA)

Specific leaf area varied based on both the origin of the propagator material as well as the location at which the plants were grown at both the initial and final sampling (Table 4.7).

The SLA was higher in the plants originating at the lower altitude at both samplings. However, the plants grown in Talawakelle showed a significantly higher SLA at the end of the experimental period.

Table 4.7 Specific leaf area according to origin of mother plants used to propagate the plants and according to location at which the plants were grown

Origin of mother plants	Specific Leaf Area (m^2kg^1)	
	Initial sampling	Final sampling
Low elevation (29m)	14.73 a	12.81a
High elevation (1382m)	12.55 b	10.64b
Location of plants		
Low elevation (29m)	15.16A	11.61B
High elevation (1382m)	13.81B	13.54A

Letters in columns within a sampling compare the variation between different altitudes. Letters indicate significant differences among responses according to origin of mother plants or location according to Duncan's multiple range test ($p<0.05$)

Leaf Weight Ratio (LWR)

The LWR was higher in the plants originating at the lower altitude at the initial sampling. The plants grown in Talawakelle showed a significantly higher LWR at both samplings.

Table 4.8 Leaf weight ratio according to origin of mother plants used to propagate the plants and according to location at which the plants were grown

Origin of mother plants	Leaf Weight Ratio	
	Initial sampling	Final sampling
Low elevation (29m)	0.38 a	0.33a
High elevation (1382m)	0.28 b	0.34a
Location of plants		
Low elevation (29m)	0.34B	0.31B
High elevation (1382m)	0.36A	0.35A

Letters in columns within a sampling compare the variation between different altitudes. Letters indicate significant differences among responses according to origin of mother plants or location according to Duncan's multiple range test ($p<0.05$)

Root Shoot Ratio

A higher root to shoot ratio was seen for the cultivars originating from the higher altitude at the initial sampling. However, the cultivars that were grown at the lower altitude showed a higher root to shoot ratio at both samplings (Table 4.9).

Table 4.9 Root shoot ratio according to origin of mother plants used to propagate the plants and according to location at which the plants were grown

Origin of mother plants	Root shoot Ratio	
	Initial sampling	Final sampling
Low elevation (29m)	0.34b	0.37a
High elevation (1382m)	0.57a	0.33a
Location of plants		
Low elevation (29m)	0.39A	0.37A
High elevation (1382m)	0.33B	0.31B

Letters in columns within a sampling compare the variation between different altitudes. Letters indicate significant differences among responses according to origin of mother plants or location according to Duncan's multiple range test ($p<0.05$)

4.3 Water stress experiment

Relative water content

The fresh weight, turgid weight and relative water content (RWC) showed a significant ($p<0.0001$) difference due to the treatment. Cultivar had a significant effect on the fresh, dry, turgid weights and RWC. A significant cultivar x treatment effect was also seen for all of the above. Fig 4.13 below depicts the cultivar based variation in RWC.

The cultivar TRI 2025 sampled from both Talawakelle and Ratnapura showed significant response to the imposed water stress. Other cultivars that showed a significant response included TRI 2026, TRI 4049 and DT-1.

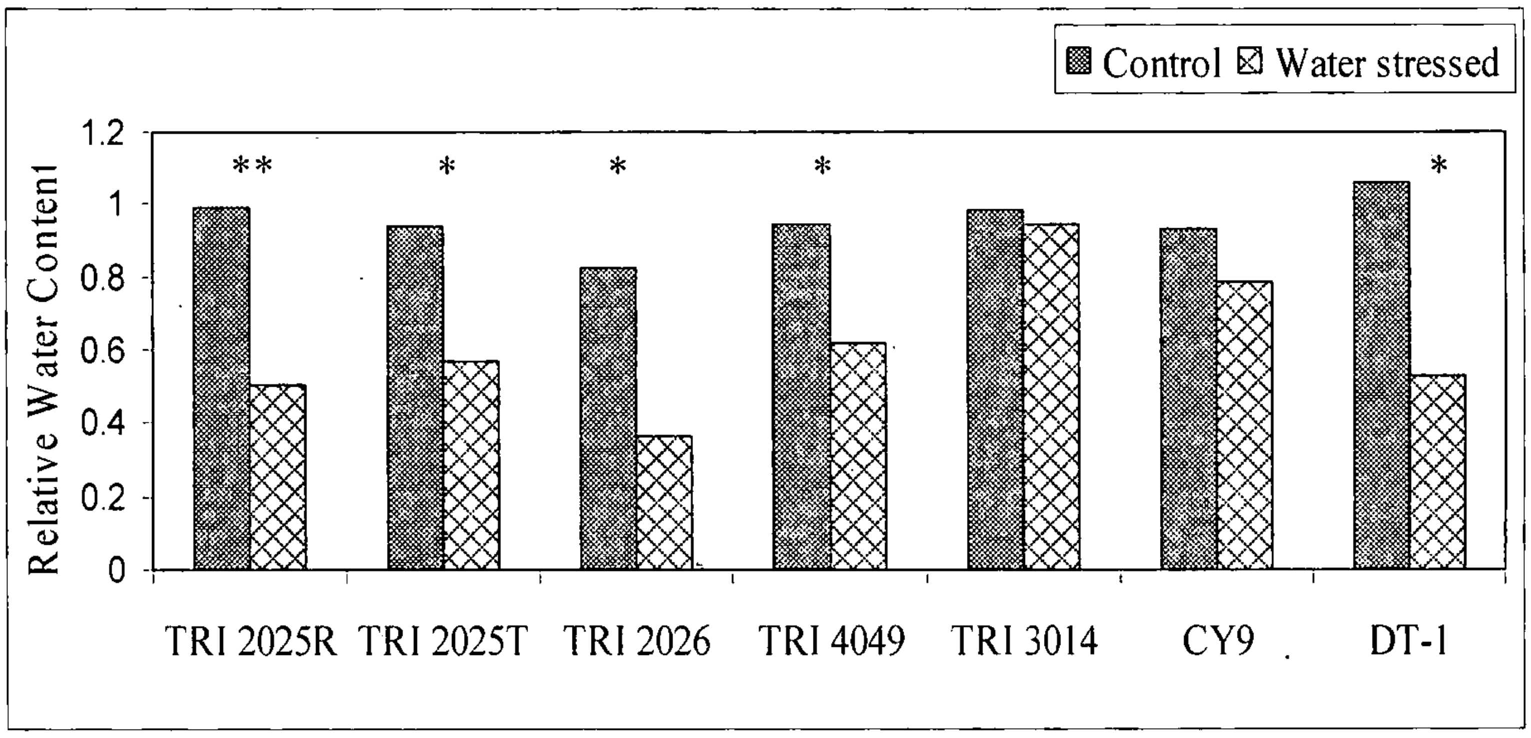


Fig. 4.13 Variation of relative water content of *Camellia sinensis* cultivars grown under control and water stressed conditions. Significant at * $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$

Plant growth studies

Analysis of variance showed that a significant treatment effect (p=0.004) and cultivar x treatment interaction (p=0.005) was present only for fresh leaf weight. However, a significant cultivar based variation was seen for the stem, root and shoot for both the fresh and dry weights. The treatment did not have a significant effect on the final dry weight of the root or shoot. Duncan grouping of the means showed that the TRI 2025 cultivars obtained from two altitudes (Talawakelle - high and Ratnapura - low) showed a similar fresh and dry weight response (grouped together) irrespective of the water treatment.

Allometric relationships

The leaf weight ratio and root to shoot ratio was calculated. The treatment did not have an impact on either of these ratios. However, a significant (p<0.01) cultivar effect was seen for the leaf weight ratio. When looking at the individual cultiavrs, TRI 2026 showed a significant response in its leaf weight ratio as it showed a higher value under the wet treatment (0.132) than the dry treatment (0.059).

The dendrogram (Fig. 4.14) obtained from average linkage cluster analysis showed four clusters which diverged from around 0.7 Euclidean distance (Table 4.10).

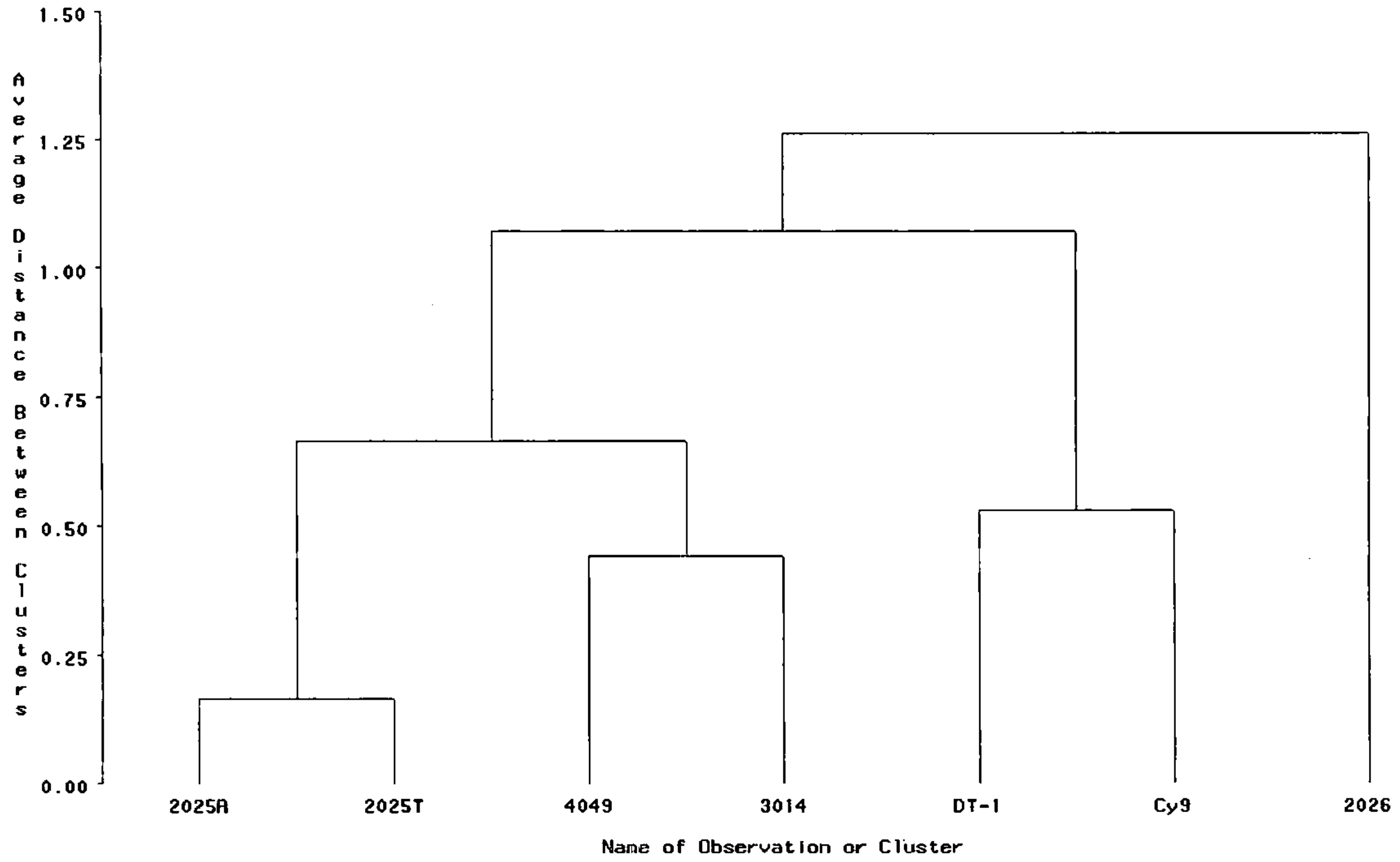


Fig. 4.14. Dendrogram obtained by average linkage cluster analysis of the relative water content and related response of *Camellia sinensis* cultivars grown under the water stress experiment

Table 4.10 The composition of clusters derived from the dendrogram

Cluster number	Cultivars within the cluster
Cluster 1	R2025, T2025
Cluster 2	4049, 3014
Cluster 3	DT-1, CY9
Cluster 4	2026

4.4 Determination of the responses of *Arabidopsis thaliana* ecotypes to elevated atmospheric [CO_2] and elevated temperature

4.4.1 Stomatal density-related characters under elevated [CO_2]

Of the 18 accessions, two accessions, namely Db-1 and Cvi-0, did not grow under elevated CO_2 treatment. Therefore, for these two, results are available only for growth under ambient conditions.

SD, ED and SI differed significantly ($p<0.0001$) between the two leaf surfaces. Hence, they are presented separately. The direction of change of SD, ED and SI in response to CO_2 enrichment and its magnitude differed significantly for different ecotypes of *A. thaliana* (Figs. 4.15 and 4.16). For example, Ba-1 showed significant reductions in all tested stomatal characters of both leaf surfaces while Col-0 showed significant increases. In contrast, ecotypes such as Su-0 and Mc-0 did not show significant variation. As a result the ecotype x CO_2 interaction effect was highly significant ($p<0.0001$).

When ecotypes were categorized based on their altitude of origin, abaxial and adaxial SD, ED and SI of different altitudinal classes again showed differential response to CO_2 enrichment. For example, all variables showed significant reductions at 550 m altitude, which was represented by the sole ecotype Ba-1. In contrast, most variables at the rest of the altitudes of origin showed increases of varying magnitudes, thus resulting in a highly significant ($p<0.0001$) treatment x altitude of origin interaction effects.

Ecotypes which showed significant increases in abaxial and adaxial SD in response to CO_2 enrichment (Figs. 4.15.a & b) represented the low (Col-0 and Pla-0), mid (Lan-0, Rsch-4, Mt-0 and Wil-2) and high altitudes (Mc-0 and Can-0). Similarly, the few ecotypes which showed significant decreases in SD also represented the whole range of altitudes. Out of the two altitudes which had higher numbers of ecotypes originating from them (i.e. 50 and 150 m), the proportion of ecotypes showing significant increases in SD in response to CO_2 enrichment were greater at 150 m than at 50 m.

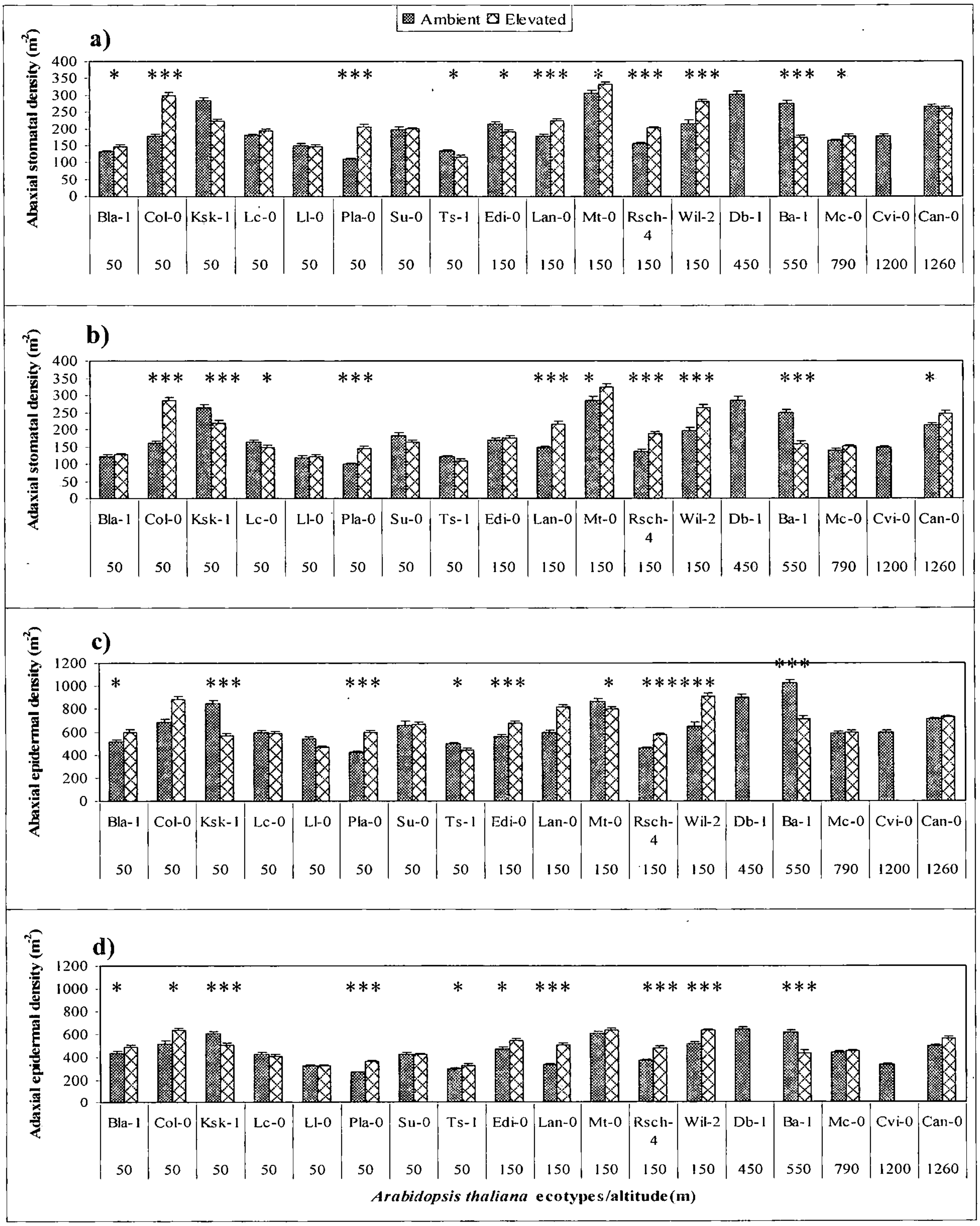


Fig. 4.15 Variation of stomatal density (SD) and epidermal density (ED) of *A. thaliana* ecotypes grown under ambient and elevated CO$_2$ treatment: a) & c) abaxial SD & ED; b) & d) adaxial SD & ED; Error bars = SE mean. n= 75. Significant at * $p < 0.05$; ** $p < 0.001$; *** $p< 0.0001$

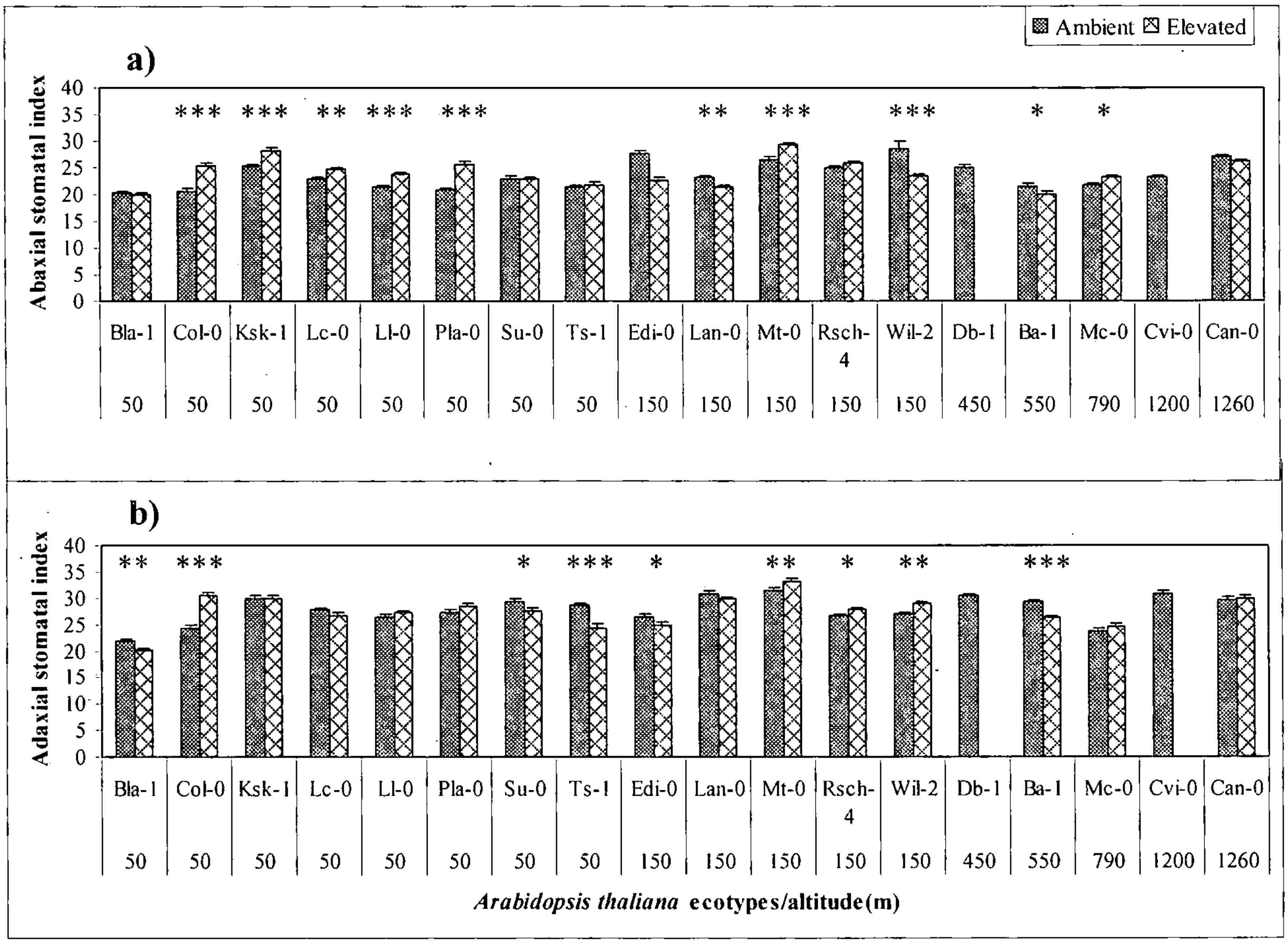


Fig. 4.16 Variation of stomatal index (SI) of *A. thaliana* ecotypes grown under ambient and elevated CO_2 conditions. a) abaxial SI; b) adaxial SI. Error bars = SE mean. *n*= 75. Significant at $*p < 0.05$; $** p < 0.001$; $*** p< 0.0001$

4.4.2 Overall response to elevated CO_2

Nine variables loaded to the first component which consisted of the SD, ED and PCI. The GCL data loaded to the second component and the SI data loaded to the third.

The dendrogram (Fig. 4.17) obtained from average linkage cluster analysis showed five clusters which diverged from around 0.8 Euclidean distance. MANOVA analysis of the clusters showed them to be significantly different from each other (Wilks' Δ=0.0029, *F* (12, 24.1) = 16.11, p<0.0001).

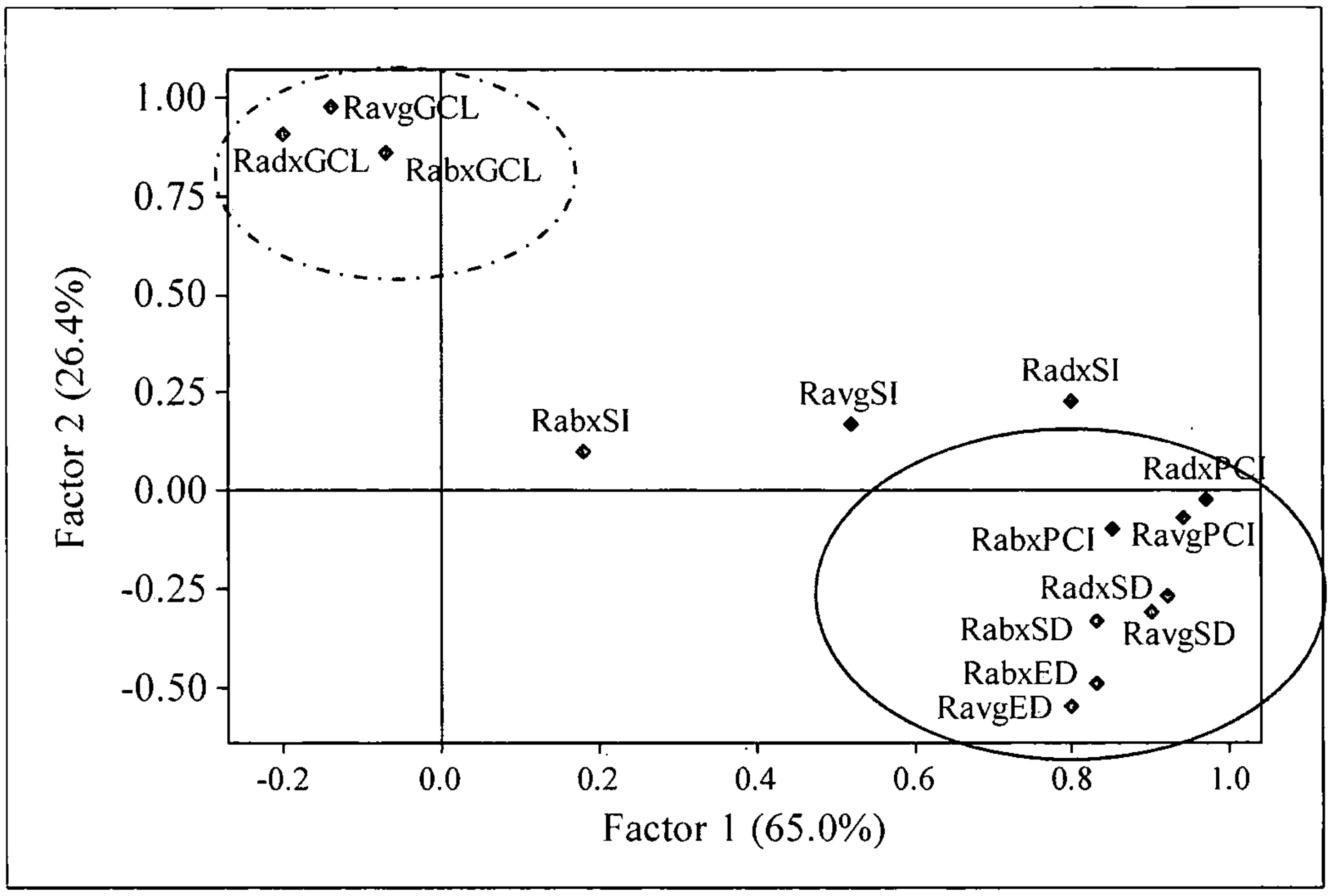


Fig. 4.17 Factor analysis for the response of stomatal characters and guard cell length data of *A. thaliana* ecotypes grown under elevated CO_2. Solid circles surround traits that loaded on to factor 1 while those circled with dashed lines indicate those that loaded to factor 2. R= response; abx=abaxial surface; adx=adaxial surface; avg=response of both surfaces; SD=stomatal density; ED=epidermal density; SI=stomatal index; GCL=guard cell length; PCI=potential conductance index

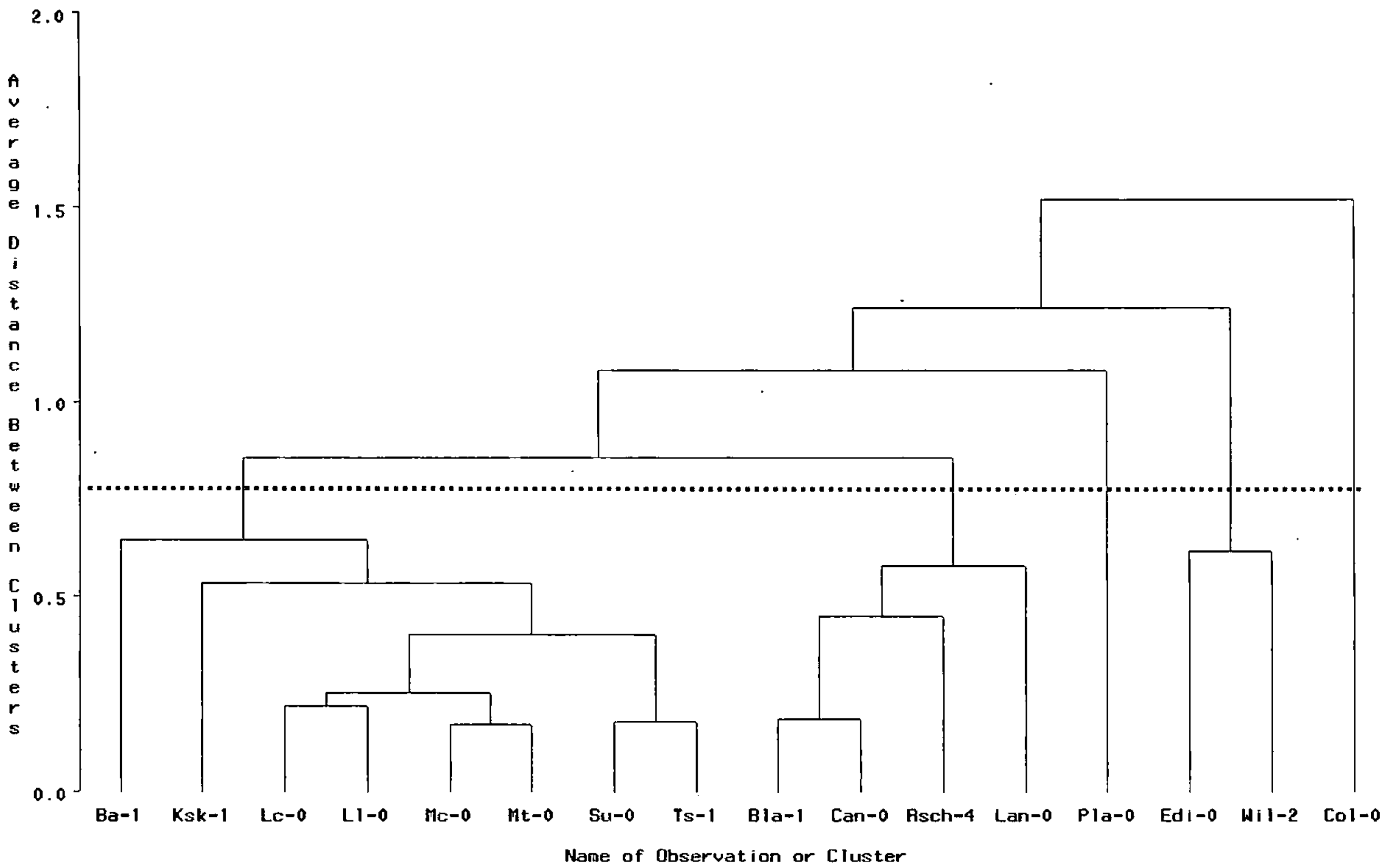


Fig. 4.18 Dendrogram obtained by average linkage cluster analysis of the response of *A. thaliana* ecotypes to growth under elevated CO_2
The cluster compositions were as follows:

Cluster 1:	Ba-1, Mt-0, Mc-0, Ksk-1, Lc-0, Su-0, Ll-0 and Ts-1
Cluster 2:	Bla-1, Can-0, Rsch-4, Lan-0
Cluster 3:	Pla-0
Cluster 4:	Edi-0, Wil-2
Cluster 5:	Col-0

4.4.3 Stomatal density-related characters under elevated temperature

Of the 18 *A. thaliana* ecotypes used for the study, it was seen that in two ecotypes (Db-1 and Cvi-0) only some of the plants survived under the elevated temperature treatment. Therefore, for these two ecotypes limited data are available under elevated temperature conditions.

A. thaliana ecotypes showed varied responses in their SD, ED and SI to elevated temperature and are presented separately according to the leaf surface. The magnitude of the change also differed (Figs. 4.19 and 4.20).

Thus, there was a highly significant ($p<0.0001$) ecotype x treatment effect. For example, both Mt-0 and Ts-1 showed a significant increase in SD and ED on both leaf surfaces. Rsch-4, Col-0 and a majority of the ecotypes showed a decrease in SD and ED on both leaf surfaces. However, certain other ecotypes such as Mc-0 did not show significant variation. SI exhibited similar ecotypic variation with increases (Col-0), decreases (Wil-2) and no significant change (Ba-1) on both leaf surfaces in response to elevated temperature. Overall SI showed a decrease with the increased temperature though this was clearly seen only on the adaxial surface.

When the ecotypes were grouped into five altitude classes based on their altitude of origin, the altitude class had an effect on the SD, ED and SI under both temperature treatments. For instance, ecotypes in the 150m altitude class showed the highest values for SD, ED and SI for both leaf surfaces under both treatments with the exception of abaxial ED. In general the lowest values for SD, ED and SI of both leaf surfaces were shown by ecotypes in the 790m or 500m altitude class. It was also seen that altitude had an effect on the magnitude of the decrease shown in SD and ED under elevated temperature treatment. Ecotypes in the 1230m altitude class showed the highest decrease in SD on both leaf surfaces in response to elevated temperature. In general, SI showed both increases and decreases for the two leaf surfaces in response to elevated temperature. The SI was observed to be less responsive than SD or ED. All three variables showed a highly significant ($p<0.0001$) treatment x altitude of origin interaction effect.

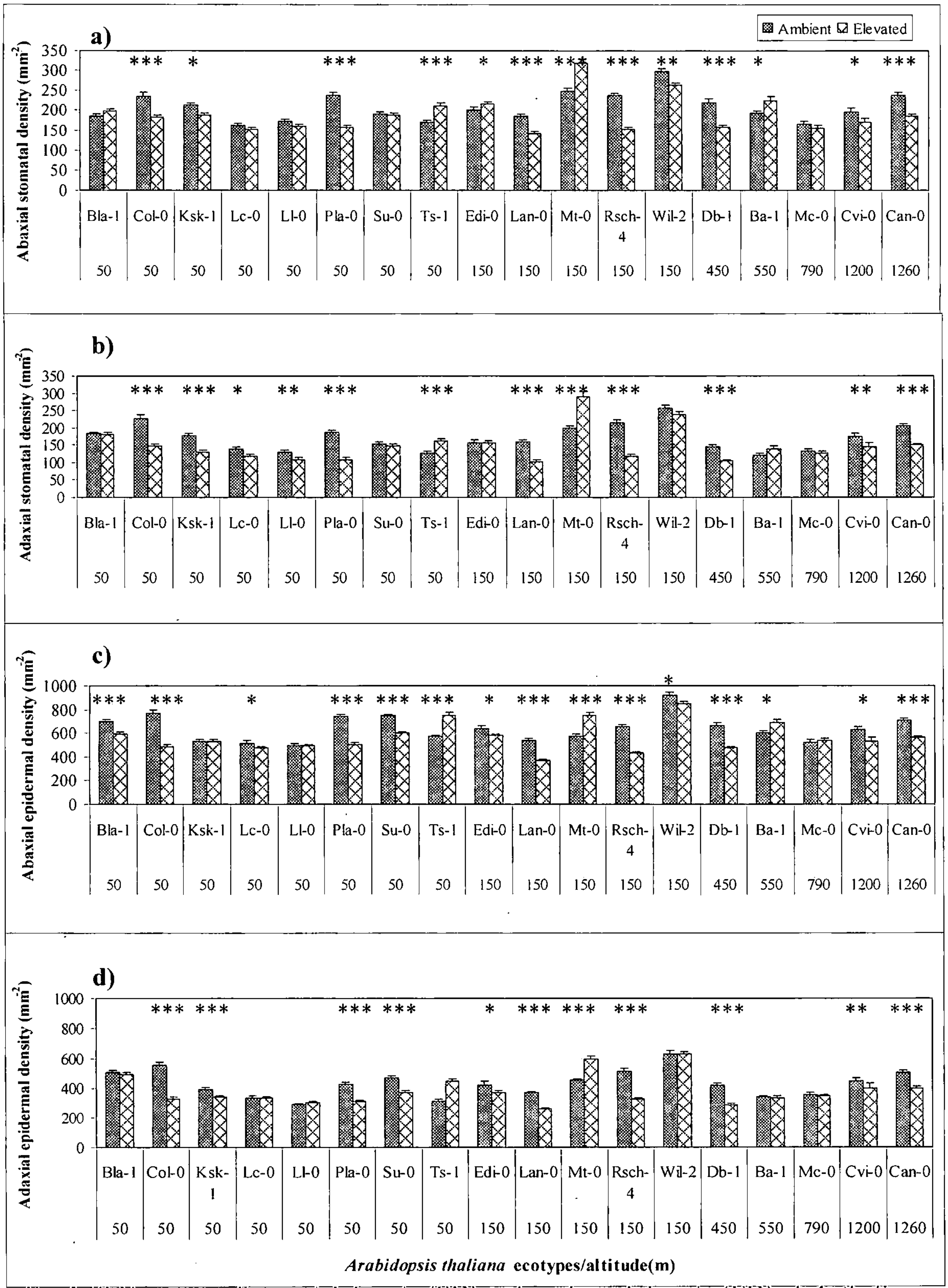


Fig 4.19 Variation of stomatal density (SD) and epidermal density (ED) of *A. thaliana* ecotypes grown under ambient and elevated temperature treatment. a) & c) abaxial SD & ED; b) & d) adaxial SD & ED. Error bars = SE mean. *n*= 75. Significant at * $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$

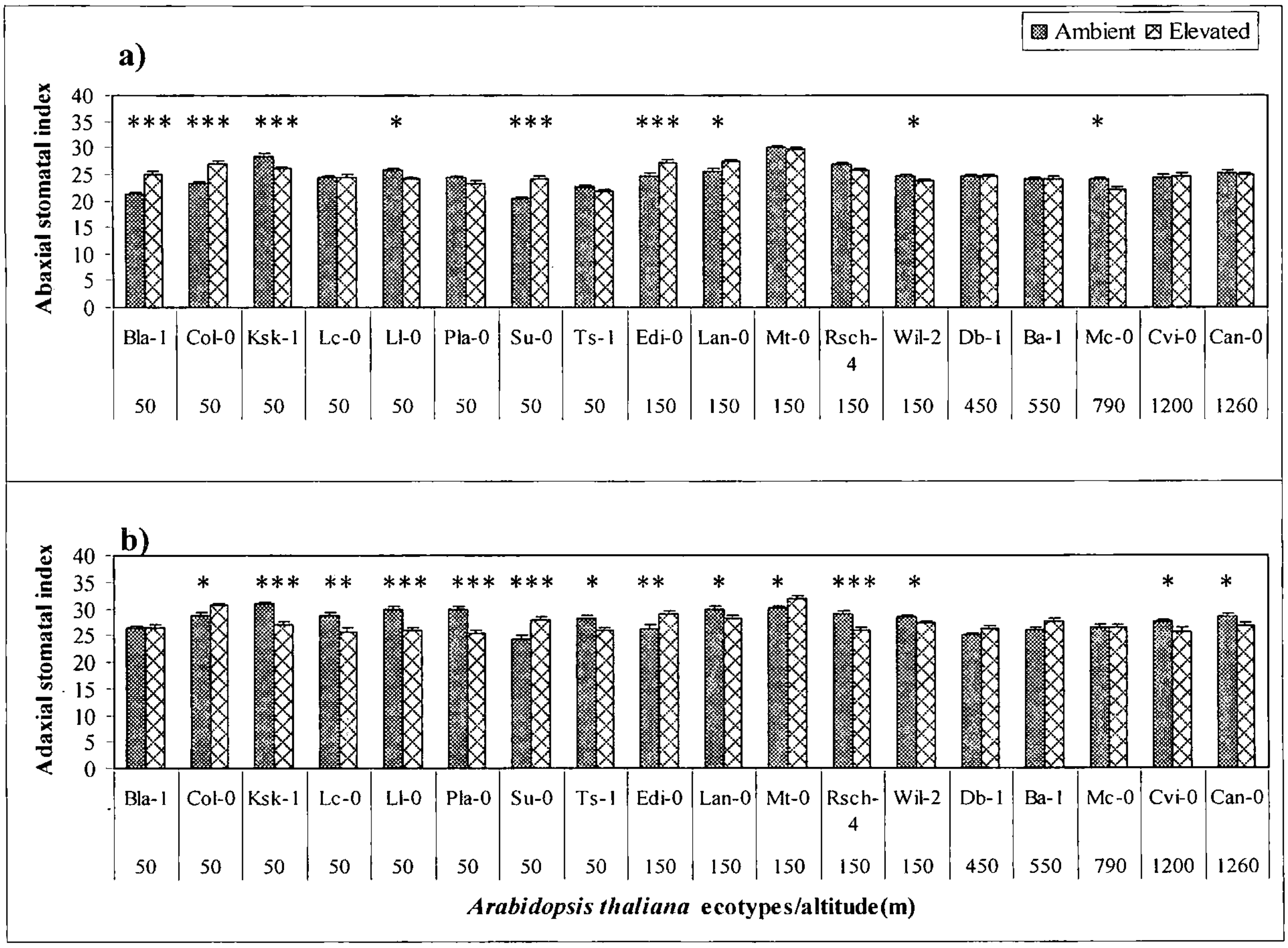


Fig. 4.20 Variation of stomatal index (SI) of *A. thaliana* ecotypes grown under ambient and elevated temperature treatment. a) abaxial SI; b) adaxial SI. Error bars = SE mean. *n*= 75. Significant at * $p < 0.05$; ** $p < 0.001$; *** $p< 0.0001$

4.4.4 Overall response to elevated temperature

Twelve variables loaded to the first factor which consisted of SD, ED, GCL and PCI. The SI data loaded onto the second factor (Fig. 4.21).

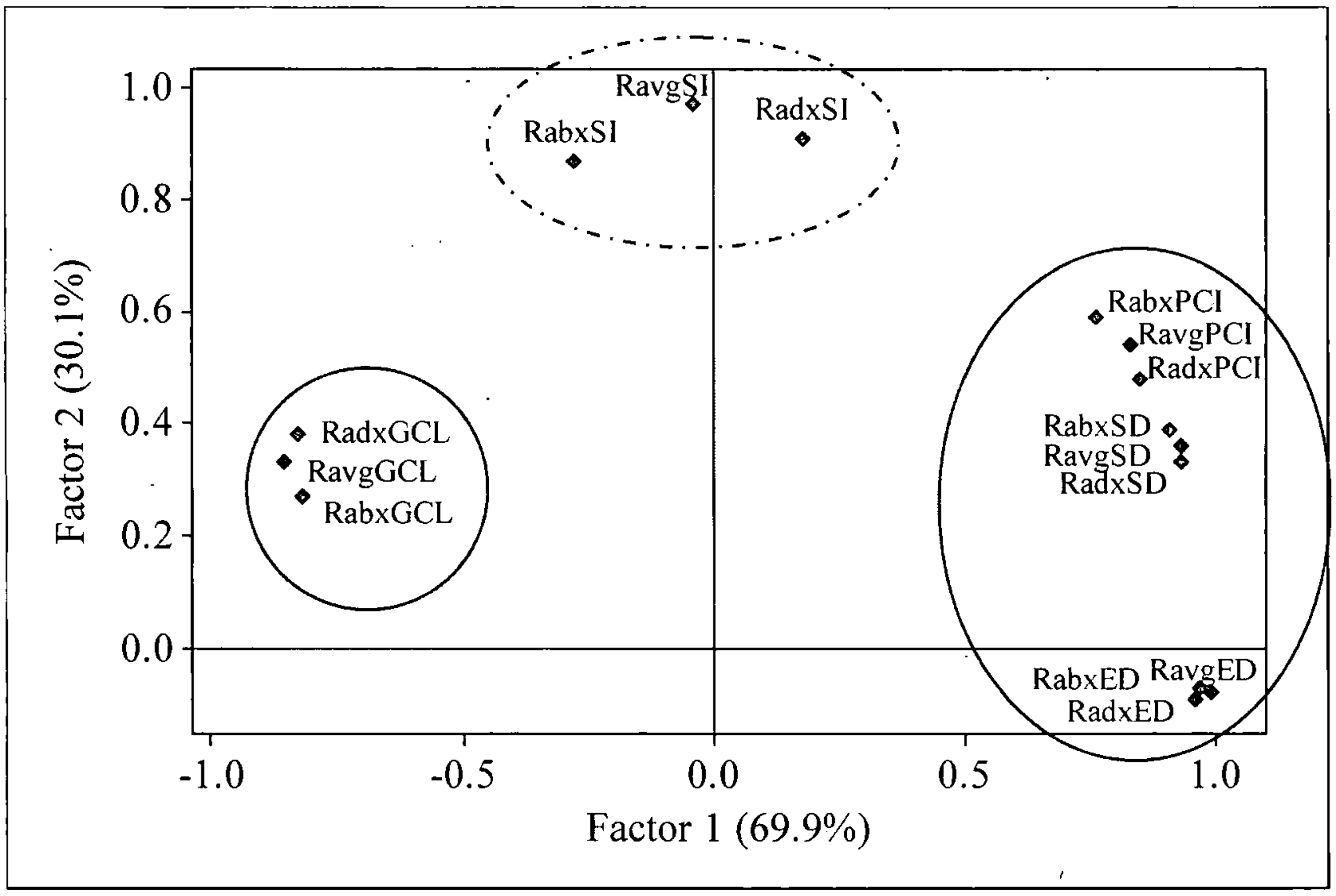


Fig. 4.21 Factor analysis for stomatal characters and guard cell length data of *A. thaliana* ecotypes grown under elevated temperature. Solid circles surround traits that loaded on to factor 1 while those circled with dashed lines indicate those that loaded to factor 2. R= response; abx=abaxial surface; adx=adaxial surface; avg=response of both surfaces; SD=stomatal density; ED=epidermal density; SI=stomatal index; GCL=guard cell length; PCI=potential conductance index

The dendrogram (Fig. 4.22) showed five clusters which diverged from around 0.7 Euclidean distance. MANOVA analysis of the clusters showed them to be significantly different from each other (Wilks' Δ=0.0143, F(8, 20) = 18.38, p<0.0001).

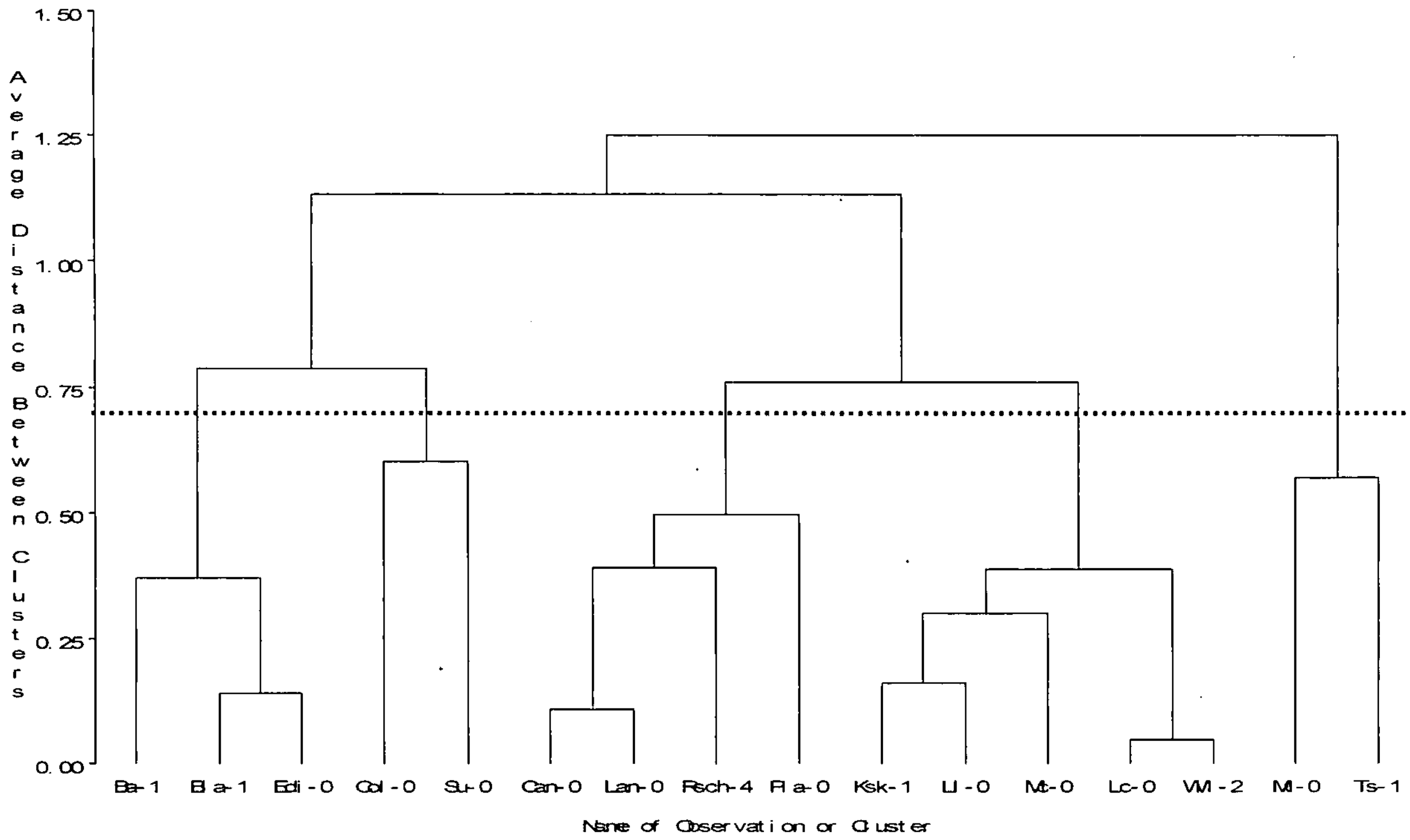


Fig. 4.22 Dendrogram obtained by average linkage cluster analysis of the response of *A. thaliana* ecotypes to growth under elevated temperature.

The clusters were as follows:

Cluster 1	A high (Ba-1) and mid (Edi-0) altitude ecotype each from UK A low altitude ecotype from Spain (Bla-1)
Cluster 2	Two low altitude ecotypes – Su-0 (UK), Col-0 (USA)
Cluster 3	The high altitude Spanish ecotype Can-0 Two mid altitude ecotypes – Rsch-4 (Russia) and Lan-0 (UK) The low altitude Spanish ecotype Pla-0
Cluster 4	The high altitude British ecotype Mc-0 The mid altitude ecotype Wil-2 (Lithuania) Three low altitude ecotypes – Ksk-1 (UK), Lc-0 (UK), Ll-0 (Spain)
Cluster 5	A mid altitude ecotype - Mt-0 (Libya) A low altitude ecotype Ts-1 (Spain)

4.5 Determination of the responses of *Camellia sinensis* to elevated atmospheric [CO_2] and elevated temperature

4.5.1. Stomatal density-related characters under elevated [CO_2]

All stomatal density-related characters showed significant spatial variation in their distribution across the leaf. However, only ED showed a significant ($p<0.05$) response to elevated CO_2 treatment. Results further showed that there was no interaction between the spatial variation and the treatment.

The SD and SI were significantly ($p<0.05$) lower at the leaf base under both CO_2 treatments (Table 4.11). Both variables increased towards the tip of the leaf though the tip and mid area values did not significantly differ from each other. Under elevated CO_2 treatment the ED did not show significant variation throughout the leaf, while under ambient conditions the ED of the leaf base was higher. While mean ED of the entire leaf increased under elevated CO_2 treatment, only the mid area of the leaf showed a significant response (3.6%). The SD and SI did not show a significant response to CO_2 treatment in any part of the leaf.

Table 4.11 The variation in stomatal density-related characters under the CO_2 treatment

CO_2 treatment	Location of stomata on leaf	SD	ED	SI
Ambient	Tip	112.0 ±2.5 aA	1001.6 ±12.2 abA	10.1 ±0.2 aA
	Mid	108.2 ±2.4 aA	984.3 ±11.0 bB	9.9 ±0.2 aA
	Base	101.1 ±2.8 bA	1028.7 ±11.7 aA	9.0 ±0.3 bA
Elevated	Tip	115.2 ±3.4 aA	1017.2 ±12.4 aA	10.2 ±0.3 aA
	Mid	112.2 ±2.9 aA	1019.7 ±13.8 aA	9.9 ±0.2 aA
	Base	100.9 ±2.7 bA	1035.9 ±10.4 aA	8.9 ±0.3 bA

Values are mean ± SE of 75 fields of view.

a-b values in columns within a given CO_2 treatment compare the variation between different leaf locations. A-B values in a given cell compare the response to elevated CO_2 within a given location of the leaf. Respective means connected by the same upper-case or lower-case letters are not significantly different at $p=0.05$ according to the Duncan's Multiple Range Test.

4.5.2 Leaf growth related traits under elevated [CO_2]

The leaf dry weight and area showed a significant ($p<0.05$) response to the elevated CO_2. The CO_2 treatment increased the leaf weight by 19.5% and the leaf area by 11% (Fig.4.23). The leaf mass per area (LMA) showed an increase of 7.4%, which was very close to being significant ($p=0.054$).

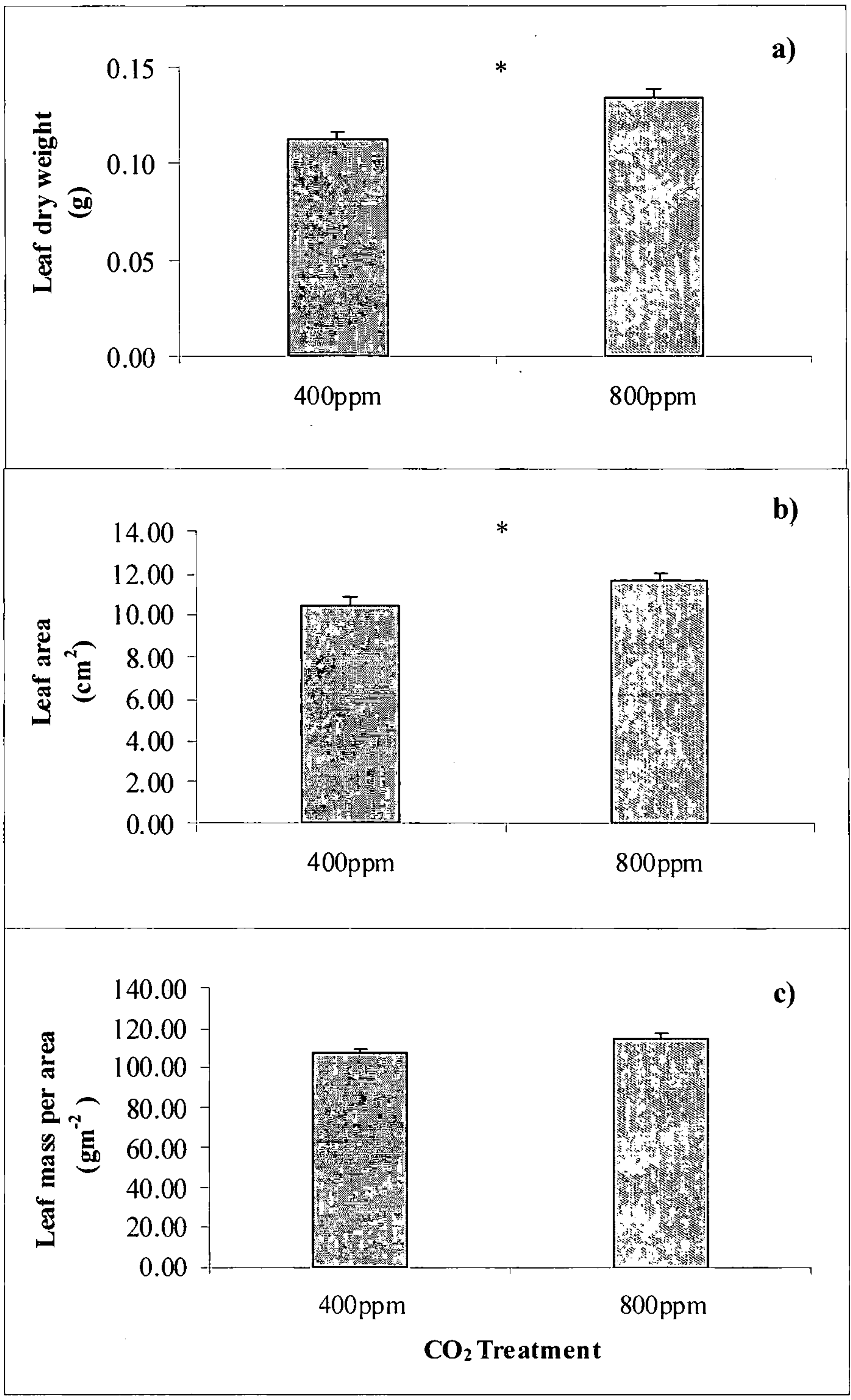


Fig 4.23 Variation of leaf dry weight (a), leaf area (b) and leaf mass per area (c) of *C. sinensis* grown under ambient and elevated CO_2 treatments. Error bars = SE mean. *n*= 15. Significant at $*p < 0.05$.

4.5.3 Leaf physiology under elevated [CO_2]

Analysis of variance showed that photosynthetic rate, stomatal conductance, transpiration rate, intercellular CO_2 concentration and instantaneous WUE were significantly different between CO_2 treatments at $p<0.0001$. Leaf temperature was significantly different at $p< 0.05$.

Net photosynthetic rate showed a 57% increase under the elevated CO_2 conditions whilst the stomatal conductance and transpiration rates decreased by 27% ad 25% respectively (Fig 4.24). Under these conditions the calculated instantaneous WUE increased by over 100% under elevated CO_2 (Fig 4.24f). The intercellular CO_2 concentration (Ci) increased by 93% and the leaf temperature decreased by almost 1% under elevated CO_2 (Fig 4.24 d & e).

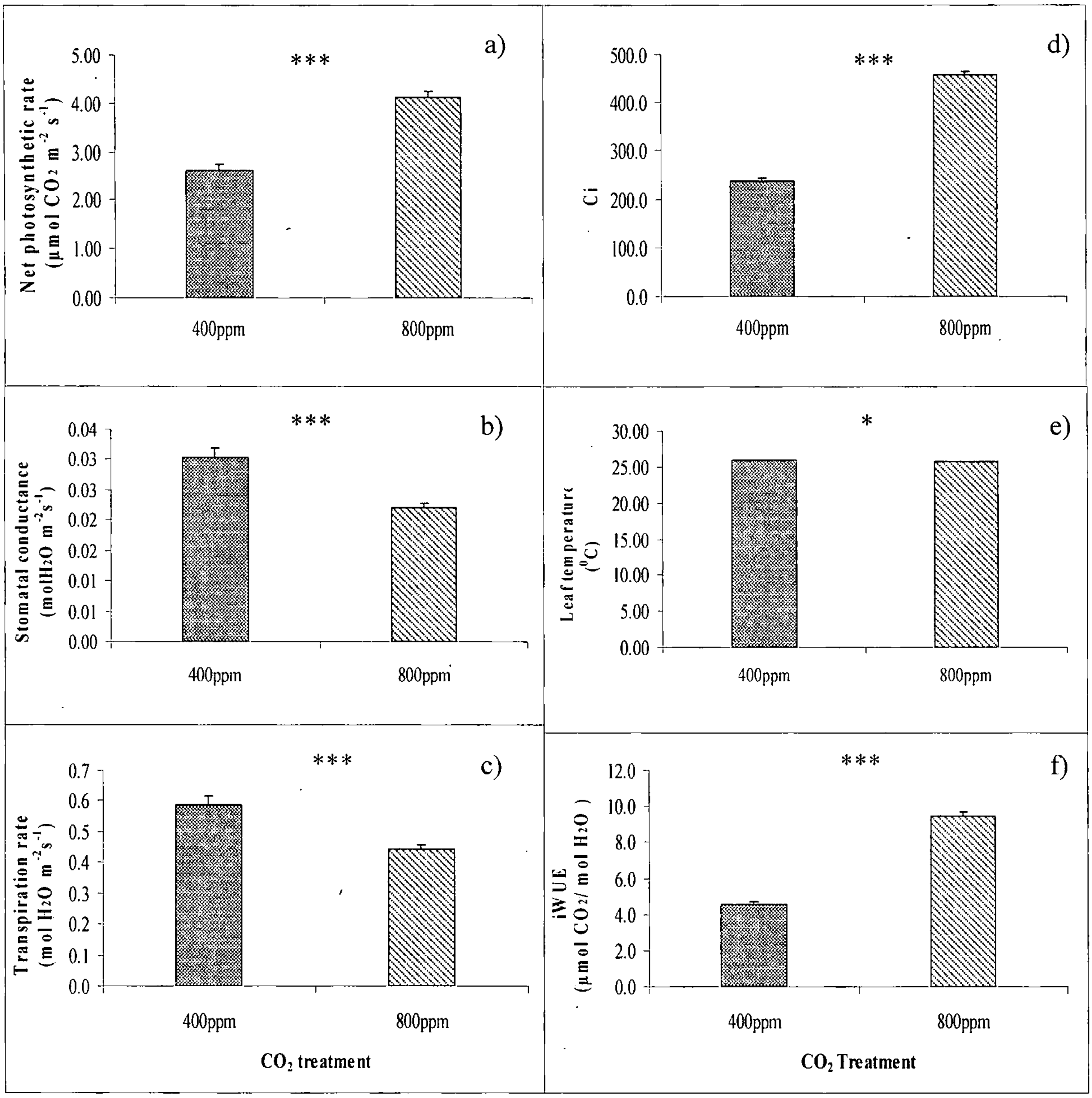


Fig 4.24 Variation in physiological parameters of *C. sinensis* plants grown under ambient and elevated CO_2 treatments. a) net photosynthetic rate; b) stomatal conductance; c) transpiration rate; d) intercellular CO_2 concentration (ci); e) leaf temperature and f) instantaneous water use efficiency(iWUE). Error bars = SE mean. *n*= 60. Significant at * $p < 0.05$; ** $p < 0.001$; *** $p< 0.0001$

4.5.4 Stomatal density-related characters under elevated temperature

All stomatal density-related characters showed significant spatial variation in their distribution across the leaf. Both ED and SI showed a significant ($p<0.0001$) response to the elevated temperature treatment. An interaction between the location of the variable on the leaf and the treatment was seen for SD and ED.

The SD and SI decreased from the tip of the leaf to the base under both temperature treatments (Table 4.12). However, a significant difference in SD and SI was seen only at the base of the leaf as the tip and mid area values did not significantly differ from each other. Under ambient conditions ED did not differ significantly with position on the leaf blade, while under elevated temperature conditions the ED of the leaf base was significantly lower. The SD at the base of the leaf and the ED across the entire leaf were significantly lower under the elevated temperature treatment (Table 4.12). The SI showed significant increases of 17% and 13% for the leaf tip and mid area respectively under elevated temperature.

When averaged across the three leaf positions, mean ED showed a significant ($p<0.05$) reduction in response to elevated temperature while SD and SI did not differ significantly. Percentage reduction of mean ED was 11% while the mean SD and SI showed 2% and 13% increases in response to temperature elevation.

Table 4.12 The stomatal density related characters based on the sampling location on the leaf

Temperature treatment	Sampling location on leaf	SD(mm^{-2})	ED (mm^{-2})	SI
Ambient	Tip	123.5 ±2.8 aA	1046.2 ±10.9 aA	10.6 ±0.2 aB
	Mid	120.3 ±2.6 aA	1048.3 ±11.6 aA	10.3 ±0.3 aB
	Base	108.2 ±2.5 bA	1040.6 ±13.2 aA	9.5 ±0.2 bA
	Mean (Ambient)	117.33±2.6	1045.03±11.9	10.13±0.2
Elevated	Tip	129.3 ±3.4 aA	921.8 ±10.4 aB	12.4 ±0.3 aA
	Mid	122.0 ±2.9 aA	928.0 ±9.6 aB	11.7 ±0.2 aA
	Base	97.3 ±2.7 bB	874.0 ±8.1 bB	10.0 ±0.3 bA
	Mean (Elevated)	116.20±3.0	907.93±9.4	11.37±0.3

Values are mean ± SE of 75 fields of view.

a-b values in columns within a given temperature treatment compare the variation between different leaf locations. A-B values in a given cell compare the response to elevated temperature within a given location of the leaf. Respective means connected by the same upper-case or lower-case letters are not significantly different at p=0.05 according to the Duncan's Multiple Range Test.

4.5.5 Leaf growth under elevated temperature

The leaf dry weight and LMA showed a significant ($p<0.05$) response to the elevated temperature treatment. Temperature treatment decreased the leaf dry weight by 9.6% and the LMA by 15.3% (Fig 4.25). Leaf area did not differ significantly in response to temperature treatment.

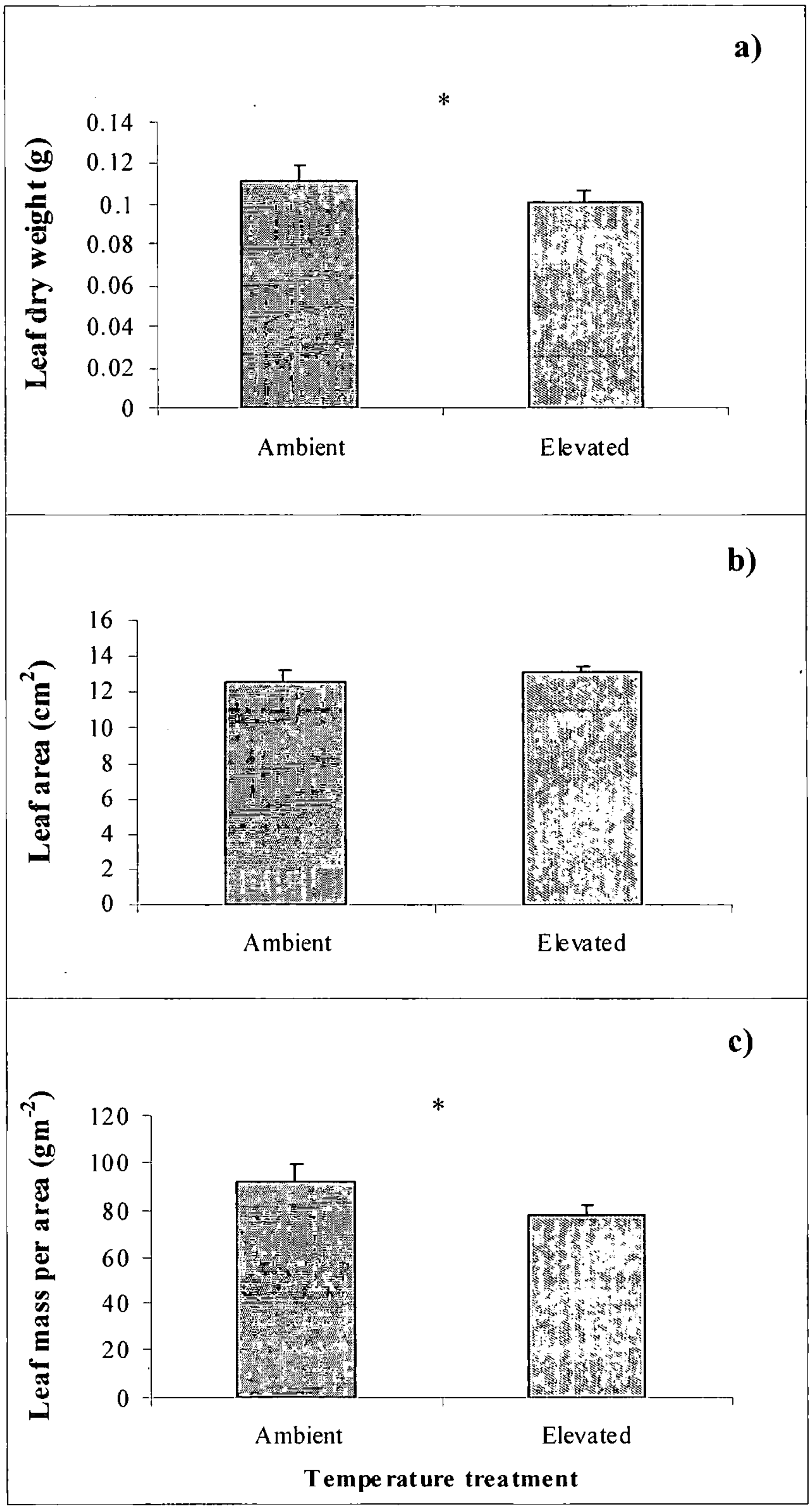


Fig 4.25. Variation of leaf dry weight (a), leaf area (b) and leaf mass per area (c) of *C. sinensis* grown under ambient and elevated temperature (21/18^{0}C and 31/28^{0}C day/night). Error bars = SE mean. *n*= 15. Significant at $*p < 0.05$.

5. Discussion

An increase in altitude represents an environmental change in terms of decreasing atmospheric CO_2 and air temperature. The observations of both increases and decreases of SD in response to increasing altitude agree with similar observations by Woodward and Kelly (1995) and Hetherington and Woodward (2003) for a wide range of natural plant species in terms of their response to increasing CO_2. Furthermore, earlier studies have shown that plants at higher altitudes show an increase in SD (Körner and Cochrane 1985; Qiang *et al.*, 2003; Kouwenberg *et al.*, 2007) as well as a decrease (Schoettle and Rochelle, 2000) in comparison to plants of the same species from lower altitudes.

The observation of a majority of tea cultivars tested showing reductions in SD with increasing altitude, i.e. with decreasing CO_2, is in direct contrast to the trend observed by Woodward (1987) and Woodward *et al.* (2002). There can be several reasons for these contrasting observations. Woodward (1993) showed that decreasing SD with increasing CO_2 conferred a competitive and selective advantage to plants by increasing water use efficiency (WUE). However, increased WUE may confer an advantage only in environments where water is a limiting factor. All three locations used in the present study are within the humid zone of Sri Lanka, known in the local classification as the wet zone, and receive an annual rainfall in excess of 1800 mm which is well-distributed within the year. Therefore, there would be no ecological advantage for the tea crops to have a higher WUE by having lower SD at the low altitude (i.e. high CO_2) and progressively increase SD with increasing altitude (i.e. decreasing CO_2). Instead, it may be more advantageous to develop higher SD at the lower altitude to increase the CO_2 absorption capacity and maximize photosynthetic rates by utilizing the higher solar radiation and temperature levels that prevail at the lower altitudes. In general, leaves exposed to high irradiation have a higher SD (*Shorea* spp. Ashton and Berlyn, 1992). Furthermore, the higher SD would be conducive to cooling leaves under the warmer conditions experienced at the lowest altitude. In contrast, the photosynthetic potential of tea crops would be lower at the higher altitudes because of lower temperatures. Accordingly, there would not be any ecological advantage for tea crops growing under the lower CO_2 levels at higher altitudes to increase their SD and increase the CO_2-suplying capacity. Therefore, results of the present study indicate that in tea, the influence of decreasing temperature in restricting photosynthetic capacity exerts a greater control in determining the variation of SD and its associated stomatal characters than the influence of decreasing CO_2 in attempting to increase the CO_2-supplying capacity. However, as demonstrated by the minority of cultivars, which have shown increases in SD with increasing altitude, the response of stomatal characters of tea to environmental change may have complex interactions with genetic factors as well as environmental factors other than temperature and CO_2. This is in agreement with the conclusions of Woodward *et al.* (2002) and Hetherington and Woodward (2003). Also, the sensitivity and the capacity to respond to environmental change may differ between different cultivars of tea. This could be the reason for the significant diversity in the response of stomatal characters to environmental change as observed in the present study.

The elevation experiment further showed that cultivars originating from Ratnapura had a higher SD and SI. Also, it was seen that SI was significantly higher in Ratnapura than in Talawakelle. Even some of the cultivars originating from the higher altitudes showed a higher SD and SI when grown in Ratnapura. As mentioned earlier with cited references, this may be in order to utilize the higher solar radiation and temperature levels that are present at the lower altitudes. The growth related studies also showed evidence for this as the RGR was significantly higher at the low altitude. In further evidence, the cultivars grown at the low altitude had a significantly higher net assimilation rate (NAR) than their up country counter parts (Table 4.6). The NAR gives the effectiveness of biomass production in terms of amount of photosynthetic tissue. It was also seen that cultivars that were grown at the lower altitude had a higher root to shoot ratio. This suggests that they cultivars when grown at the lower altitude have a higher root growth and this may be necessary as there is a higher chance of lack of water at Ratnapura rather than at Talawakelle. It may be that the cultivars have evolved to maximise their photosynthetic ability by provision of all necessary inputs.

However, it should be noted that there was a cultivar diversity to be seen and this further shows the response diversity of the local tea cultivars.

Looking at the stomatal conductance values it was seen that the highest stomatal conductance was shown by the cultivars growing at the lowest altitude. In fact it was observed that irrespective of where the plants were propagated from, cultivars growing at Ratnapura, i.e. the lower elevation had a significantly ($p<0.001$) higher stomatal conductance. Other than that cultivars propagated from the lower altitude had a significantly higher stomatal conductance than those propagated from the higher altitude which in turn points to a genetic basis as well. This agrees with the earlier seen higher SD values as a higher number of stomata generally leads to a higher degree of conductance. Broad leaved herbaceous crops are generally reported to have a stomatal conductance above 500mmolm^{-2}s^{-1} (Körner,1994). This is because they are fertilised and have undergone selective breeding for productivity leading to a higher conductance value

It was also seen that cultivars originating from the lower altitude showed a higher leaf area than those from the higher altitude for both the initial and final sampling in the elevation experiment. A higher leaf area would support a large number of stomata as well, leading further evidence to the higher stomatal conductance observed at the lower altitude. The fact that this was seen for cultivars originating from the lower altitude, irrespective of where they were grown points to a genetic basis for the larger area at the lower altitude.

With regard to variation in SD with altitude it is also possible that tea is not responding to the CO_2 gradient across the altitudinal range as it may be a plant that is insensitive to this environmental parameter with regard to stomatal numbers. Earlier studies on stomata have shown that some species are insensitive to changes in CO_2 concentration (Radoglou and Jarvis 1990; Ryle and Stanley, 1992; Estiarte *et al.*, 1994; Case *et al.*, 1998; Poole *et al.*, 2000; Reid *et al*, 2003) and it may be that tea follows this trend. Additionally, the controlled environmental studies showed that stomatal numbers in tea did not respond to elevated CO_2 further supporting the lack of a stomatal response of tea to a CO_2 gradient. The growth chamber studies on elevated temperature on tea showed that SI increased under the elevated temperature treatment. This shows that stomatal development itself was affected by the temperature treatment. The lower altitude in the present study had the highest temperature in comparison to the other locations. Taken together with the results of the elevated temperature growth chamber study this suggests that tea stomata are responding to the variation in temperature over that of the CO_2 gradient. Earlier studies have also pointed to higher stomatal numbers under elevated temperature (*Lolium* *perenne*, Ferris *et al.*, 1996; *Gossypium hirsutum*, Reddy *et al.*, 1998; *Carex* spp. Stenstrom *et al.*, 2002; *A. thaliana,* Jin *et al.*, 2011).

The cultivars TRI 3014 and TRI 4053 showed the highest degree of responsiveness to environmental change as they showed significant variation in all stomatal variables measured. Therefore, these cultivars have the genotypic potential to alter both their stomatal numbers and dimensions in response to future climate change scenarios. Conversely cultivars such as TRI 2043 show an overall limited response to varied environments. Thus, the Sri Lankan tea cultivars may show varied responses in their stomatal characters to future environmental change. Such diversity of responses to future climate change within the Sri Lankan tea germplasm would be a significant advantage in breeding new cultivars which are better adapted to future climates.

The tea cultivars showed an increase in leaf thickness with increasing altitude. Plants from higher altitudes generally have thicker leaves than their lowland counterparts (*Nothofagus menziesii*, Körner *et al.* 1986; *Metrosideros polymorpha*, Cordell *et al.*, 1999; *Polygonum cuspidatum*, Kogami *et al.* 2001; rain forest tree species; Velázquez-Rosas *et al.* 2002; *Fagus sylvatica* & *Quercus petrea*, Bresson *et al.* 2011). The tea cultivars grew under a temperature gradient with the plants at the highest altitude experiencing the lowest temperature. Studies by Chabot and Chabot (1977) on *Fragaria vesca* have shown that experimentally induced cold temperatures lead to thicker leaves with poor internal development. Temperature exerts a strong control over leaf thickness and most altitude based studies have attributed thicker leaves to the lower temperature at higher altitudes (Woodward, 1979; Körner *et al.* 1986; Woodward 1983). Cordell *et al.* (1998) showed through complementary field and common garden experiments that changes in leaf thickness was largely induced by the environment and not the genetic background. Thus, the increase in leaf thickness of the tea cultivars with altitude can be attributed to the temperature gradient that accompanies the altitude gradient.

Thicker leaves may lead to an increase in photosynthetic capacity due to the larger number of chloroplasts and the increased amount of enzymes of CO_2 assimilation (Körner and Diemer, 1987; Niinemets, 1999; Evans & Poorter, 2001). However, an increase in leaf thickness may be a disadvantage for CO_2 uptake as the distance between the stomata and the carboxylation site increases (Vitousek, 1992). It is generally regarded that allometric ratios amongst mesophyll tissues are more important in evaluating plant responses than merely measuring the leaf thickness (Pritchard *et al.*, 1999). Leaves sampled from the lowest elevation, Ratnapura showed the highest palisade:spongy parenchyma ratio (PSR) and palisade to total leaf ratios and the lowest spongy parenchyma to total leaf thickness ratio. In general, plants growing under increased irradiance have a thicker palisade layer and a diminished spongy layer (Ashton and Berlyn, 1992). The palisade layer acts as a light conduit which propagates light deeper into the mesophyll and thereby evenly distributes light within the leaf (Smith *et al.*, 1997). A palisade rich mesophyll is therefore associated with a high area based gas exchange rate and studies have shown that light saturated photosynthetic rate is influenced more by the palisade layer thickness than the overall leaf blade thickness (Kenzo *et al.*, 2004). In most leaves the palisade surface is greater than that of the spongy layer with the former in addition containing more chloroplasts. For instance, studies on *Camellia japonica* have shown that the chlorophyll concentration of the palisade layer was 1.5 – 2.5 times greater than that of the spongy tissue (Terashima and Saeki, 1983). Thus, most of the photosynthesis occurs in the palisade layer (Raven *et al.*, 2005) and high photosynthetic rates are seen for the adaxial leaf surface (Peguero-Pina *et al*; 2009). Hence, the cultivars growing in the lowest elevation (Ratnapura) may have adapted to make maximum use of the abundant resources of high sunlight and adequate rainfall as shown by the increased thickness of the palisade layer in relation to the entire leaf.

Wickramaratne (1981) studied genotype × environment interactions in tea and measured productivity using the yield in fresh weight averaged over a 6-7 year period. Based on the results the study environments were quantitatively graded for productivity as Mid country wet zone (Hantane) < Up country wet zone (Talawakelle) < Low country wet zone (Kottawa). Kottawa at 30m amsl is at a similar elevation and in the same agro-ecological zone as Ratnapura and therefore, can be taken as a proxy for the latter. The grading for productivity of the above study ties in with the separation of means for palisade layer ratios in the present study.

The estate cultivars from each altitude consistently showed a lower PSR and palisade to total leaf thickness ratio in comparison to newly developed TRI cultivars. Based on the literature discussed above, this suggests that these cultivars may have a lower productivity than the newly developed TRI cultivars. Analysis of the TRI advisory circular on cultivar recommendations shows that 82% of the newly recommended cultivars are regarded as high yielding cultivars as opposed to only 50% of the estate cultivars (Anon. 2002). While all the cultivars in this circular were not subjected to the study, it is of note that all cultivars in the present study were selected from this TRI document. Furthermore, early field trials using TRI cultivars (TRI 2025, TRI 2026 and TRI 2027) and estate cultivars (DT-1 and CY9) across a range of altitudes had shown that the yield was higher in the TRI cultivars (Richards, 1967). Thus, it is plausible that the lower palisade tissue content in the estate cultivars is contributing to the relatively lower yield reported in literature.

The cultivars that were grouped close to the estate cultivars both in the scatterplots as well as under Duncan's mean separation procedure included TRI 2043, TRI 4049, TRI 4053, TRI 3016 and TRI 3019. It is interesting to note that all of these cultivars except for TRI 2043 were derived from an Assam/Cambod cross with an estate cultivar. (TRI 4049 and TRI 4053 from a cross with CY9 and TRI 3016 and TRI 3019 from a cross with DT 95). TRI 2043 too is of Indo-Chinese origin and not the progeny of an improved cultivar developed by the TRI. This grouping together of estate cultivars as well as newly developed cultivars originating from the estate cultivars further shows that the characters discussed here are inherent in these plants.

The specific leaf area (SLA) was calculated for the cultivars used for the elevation experiment. Here it was seen that SLA was higher at the lower altitude and lower at the high altitude. SLA is an indirect measurement of leaf thickness as it represents the leaf area over which a unit leaf dry weight is spread. A higher SLA is indicative of thinner leaves and vice versa. Thus the results obtained from the elevation experiment supports the observations mentioned above for the mature tea bushes, i.e. thicker leaves were observed at the high altitude.

Factor analysis showed that the measured variables loaded onto four factors with factors one and three consisting of anatomical characters while components two and four had the stomatal related data. The leaf anatomical allometric ratios loaded to component one and thus explained a majority of the variation.

In the dendrogram (Fig 4.8), the estate cultivar PK-2 sampled from Hantane and TRI 2026 from Ratnapura, diverged early on from the other cultivars. PK-2 sampled from Hantane was the only cultivar in the study that was growing under shade. Univariate analysis showed that it had anatomical and stomatal characters that were ($p<0.05$) significantly different from the other cultivars. For instance, it showed the lowest leaf thickness, PSR and palisade to leaf ratios amongst all the cultivars, which are typical anatomical characteristics of shade leaves (Ashton and Berlyn, 1992; Smith *et al.*, 1997). TRI 2026 which was sampled only from Ratnapura had the overall highest PSR and palisade to leaf ratio along with the lowest spongy to leaf ratio and these characters would have led it to diverge from the other cultivars.

Cluster number one included TRI 2025 sampled from all three altitudes as well as the cultivar TRI 2027, sampled from mid and low elevations. TRI 2025 and TRI 2027 are cultivars that yield well at all locations and demonstrate a general adaptation (Wickramaratne, 1981). The stomatal and anatomical characters support the above as these two cultivars show similar characters at all elevations. Cluster number one also included TRI 3014 and TRI 3055 sampled from both low and high elevations. This suggests that TRI 2025, TRI 2027, TRI 3014 and TRI 3055 show a limited response to the environmental parameters experienced in the locations from which they were sampled as the phenotype of these cultivars exhibits similar stomatal and anatomical characteristics irrespective of the growth environment.

TRI 2025, TRI 2027 and TRI 3055 share the same origin as they are progenies from open pollination of the Assam/Cambod introduction. TRI 3055 is the progeny of open pollination of TRI 2025. Hence, this cluster consists solely of cultivars which are derived from the Assam/Cambod introduction.

Cluster number two mainly consisted of cultivars sampled from the mid and high elevations. A majority of the cultivars in this cluster were estate cultivars or cultivars derived from crosses between estate cultivars and the Assam/Cambod introduction. It included all the estate cultivars that were sampled from the mid country – CY-9, KEN 16/3 and DN (except PK-2 sampled in the shade). It also included TRI 3019 and TRI 3016 which are progeny of a cross between DT 95 and the Assam/Cambod introduction, along with TRI 4053 and 4049 which are derived from a CY9 and Assam/Cambod cross. TRI 2043 which was the only cultivar in the study that was of an Indo Chinese origin was also in this cluster. TRI 2016 and TRI 3025 sampled in the low country, which are a progeny from the open pollination of the Assam/Cambod introduction and the open pollination of TRI 2025 respectively, were two cultivars in this cluster that did not have an estate cultivar origin. TRI 2043 and TRI 3019 sampled from both mid and high elevations showed similar characteristics and clustered together. Thus, these two cultivars also show a limited response to the changes in environmental parameters across the elevation gradient.

However, in the case of TRI 3025, TRI 4049 and TRI 4053, the cultivars sampled at the low and high elevations clustered separately. These results show that the cultivars respond to the location in which they are grown by varying their stomatal and anatomical characters. TRI 4049 is recommended specifically for the lower elevations (Anon, 2002) and this may have led it to show dissimilar characteristics when sampled from the low and high elevations. Conversely, TRI 4042 sampled at both high and low elevations clustered together in cluster number four, indicating that this cultivar shows a limited response to environmental variability.

Cluster number three consisted solely of cultivars sampled at the lower elevation and also included all the estate cultivars sampled from the low country (H1/58 and KEN16/3). As TRI 4049 and TRI 4053 are derived from CY9, all four cultivars in cluster three had an estate cultivar origin.

All the estate cultivars sampled at the higher elevation (CY9, DN, PK-2 and DT-1) formed the fifth cluster. Overall, the dendrogram clearly demonstrated that estate cultivars clustered according to the altitude from which they were sampled. This suggests that the environmental influence on their stomatal and anatomical response is greater than the influence from the genetic background. Similar results were obtained for anatomical characters of *Metrosideros polymorpha* across an altitudinal gradient (Cordell *et al.*, 1998). Thus, the estate cultivars sampled in this study are highly responsive to their growth environment in comparison to the mixed result obtained for the newly developed cultivars.

Ten cultivars developed by the TRI were sampled at more than one elevation in this study. Out of these ten, seven cultivars clustered with its counterpart from a different location. Three of these cultivars (TRI 2025, TRI 2043 and TRI 4042) were those recommended for growth at all three elevation levels (Anon, 2002). Therefore, they would be cultivars that show general adaptation to the environment. Another two cultivars out of the seven, TRI 3019 and TRI 2027 were recommended for two elevation levels each and were sampled from these specified elevations. Therefore, they too would show a general adaptation.

As tea is cultivated as a rain fed perennial crop water stress acts as critical factor that determines tea productivity in certain parts of Sri Lanka (Karunaratne *et al.*, 1999). Also, young tea plants are more prone to depletion of soil water availability than mature bushes due to the younger and a shallower root system. Thus, pot plant based water stress experiments using young tea plants are useful to evaluate results. Studies suggest that low country tea plantations are more prone to adverse effect on climate change than up country tea plantations (Wijeratne *et al.*, 2007) and hence the present study focused on using cultivars mostly obtained from the lower altitude of Ratnapura.

The cultivars under study showed a significant decrease in Relative Water Content (RWC) which is a commonly seen drought response in many species (Hossain *et al.*, 2010; Salekjalali *et al.,* 2011; VidhanaArachchi *et al.,* 2012; Hernandez *et al.*, 2013). Previous studies on tea has shown that depletion of soil water availability reduce RWC in tea leaves (Upadhyaya and Panda, 2013; Damayanthi *et al.*, 2010). In the present study TRI 3014 and CY9, showed a limited change in its RWC under water stress treatment. Higher RWC has been reported to play a role in the stress tolerance of plants (Damayanthi *et al.*, 2010) and to be a good indicator of drought stress tolerance (Shaw *et al.*, 2002). CY9 for instance is a known drought tolerant cultivar with a deeper root network (Damayanthi *et al.*, 2010). Both CY9 and TRI 3014 are classified as drought tolerant cultivars as well as high rooting ones by the TRI (Anon, 2002).

The leaf weight ratio was significantly different for TRI 2026 due to the treatment. This would be due to the high leaf fall seen in this cultivar compared to the other cultivars in the present study. Significant results were not obtained for allometric relationships such as root to shoot ratio and this may be due to the short period of time allocated for the pot plant experiment.

The dendrogram (Fig. 4.14) obtained from average linkage cluster analysis showed four clusters which diverged from around 0.7 Euclidean distance. TRI 2026 which is a known drought susceptible cultivar diverged early on in the clustering process (Anon, 2002). Also, in terms of RWC it showed the lowest value amongst the cultivars under both treatments (Fig. 4.12). TRI 2025 cultivars obtained from both Ratnapura (2025R) and Talawakelle (2025T) showed a similar response and clustered together. This suggests that this cultivar shows a related drought response in terms of relative water content irrespective of altitudinal origin. The two estate cultivars used in the study (CY9 and DT-1) clustered together in the third cluster.

The cultivars in the second cluster, TRI 4049 and TRI 3014 are both from the Assam/Cambod introduction as was the 2000 series cultivar, TRI 2025 which clustered close by.

UK based studies

The *A. thaliana* ecotypes showed a wide range for the measured parameters under ambient CO_2 as well as in their response to elevated CO_2. A similar result was seen under the temperature experiment. The ecotypes originate from diverse locations and have evolved under varying temperature regimes. Therefore, they would have been subjected to diverse selective pressures in their native locations. Thus, a significant ecotypic variation at ambient temperature and a significant ecotype based interaction with the treatment (elevated temperature) for the different variables is an expected result. In *A. thaliana* stomatal development, the number of amplifying divisions that regenerate meristemoid and sister cells is ecotype dependent (Casson and Gray, 2008). These cells finally develop into stomata and subsidiary epidermal cells. Thus, this amplifying division step may cause the ecotype based diversity seen in stomatal and epidermal numbers under all treatments.

Most of the ecotypes in the present study showed an increase in SD in response to elevated CO_2 though the typical response shown by many species grown under elevated CO_2 is a decrease in SD (Woodward, 1987; Clifford *et al.*, 1995; *Olea europaea*, Tognetti *et al.*, 2001) and many studies have reported this response for *A. thaliana* as well (Woodward *et al.*, 2002; Hetherington and Woodward, 2003; Teng *et al.*, 2006). The varied response to elevated CO_2 demonstrated in the present study maybe due to the response diversity that is inherent in *A. thaliana* ecotypes. It should also be noted that the decrease in SD under elevated CO_2 that is commonly recorded in studies is not a universal response. Some studies have noted an increase in SD or SI under elevated CO_2 (*Phaseolus vulgaris*, O'Leary and Knecht, 1981; *Quercus robur*, Atkinson *et al.*, 1997; *Alnus glutinosa,* Poole *et al.* 2000; *Solanum tuberosum,* Lawson *et al.*, 2002). A meta-analysis of SD responses under free-air CO_2 enrichment (FACE) experiments showed that the average 5% decrease in SD was not statistically significant (Fig. 2 of Ainsworth and Rogers, 2007). The authors reasoned that responses such as decreased stomatal conductance under elevated CO_2 was probably due to changes in stomatal aperture and not SD. In fact, Lawson *et al.* (2002) state that there may not be a need to alter SD in response increased CO_2 as the aperture size may provide effective regulation.

A significant ($p<0.0001$) altitude of origin effect was seen for SD and the SD increased with increasing altitude under both ambient and elevated CO_2 conditions. Previous studies have shown a similar diversity with high altitude plants having a higher SD than their lowland counterparts (Körner and Cochrane 1985; Woodward *et al.*, 2002, Qiang *et al.*, 2003). This effect is believed to be caused by the lower CO_2 partial pressure experienced at higher altitudes (Gale, 1972). A higher SD would act to increase the range of stomatal conductance at the higher altitudes, leading to an increase in photosynthetic rate under the relatively limited CO_2 environment (Woodward *et al.*, 2002). It is plausible that diverse geographical locations and varied local environments from which ecotypes originate influence the SD response over the influence of altitudinal range. Xie *et al.* (2009) and Luo *et al.* (2006) saw a similar result where SD did not vary in a linear manner with altitude in *Gingko biloba* and *Picea asperata* populations from a diverse altitudinal range. The different climatic conditions at sampling sites (Xie *et al.*, 2009) and genetic adaptation to the native habitats (Luo *et al.*, 2006) were given as possible reasons for the results.

An increase in SI with increasing altitude was seen in the present study, especially on the abaxial surface. While previous studies have established this trend for SD (Körner and Cochrane 1985; Woodward *et al.*, 2002, Qiang *et al.*, 2003) most altitude based studies have not determined the SI of the study species. However, studies on SI are of importance as they reveal whether stomatal cell division and differentiation were affected by CO_2 treatment (Morison, 1998). Overall it was seen that the abaxial SI was more responsive than the adaxial. This may be due to higher initial number of stomata on the abaxial surface being more responsive to elevated CO_2 than the lower number seen on the adaxial surface (Woodward and Kelly, 1995; Casson and Gray, 2008). Also it may be due to differing environmental conditions on the respective leaf surfaces such as light (Ceulemans *et al.*, 1995) and intrinsic characteristics of the two surfaces (Morison, 1998).

In terms of elevated temperature most of the ecotypes showed a decrease in SD of both leaf surfaces under elevated temperature treatment. This is in agreement with a number of earlier studies on the influence of high temperature on stomatal parameters (Luomala *et al.*,2005; Beerling and Chaloner, 1993; Ferris *et al.*, 1996). However, varying results such as an increase in SD (Ferris *et al.*, 1996; Reddy *et al.*, 1998; Jin *et al.*, 2011) and no change (Ciha and Brun, 1975; Apple *et al.*, 2000; Kouwenberg *et al.*, 2007) have also been reported. In the present study, a limited number of ecotypes showed increases as well as no significant change in SD under elevated temperature. While previous studies have suggested the equivocal results to be due to species based differences, the present study suggests that intraspecific differences such as those seen amongst ecotypes could also be a likely cause. Furthermore, the magnitude of the temperature treatment may also play a role (Jin *et al.*, 2011). Fewer stomata potentially means a reduction in water use when compared to the same leaf area with more stomata (Ciha and Brun, 1975; Luomala *et al.*, 2005). Apple *et al.* (2000) reported no change in SD of *Pseudotsuga menziesii* saplings under elevated temperature though stomatal conductance decreased. The previous studies suggest that aperture level control has a greater impact on the stomatal diffusion of water under elevated temperature and it is plausible that this applies to *A. thaliana* ecotypes studied here.

A lower SD could be the result of a decrease in stomatal formation, an increase in size and/or number of epidermal cells or a combination of both. However, the results showed that ED also significantly decreased under elevated temperature. Leaves developing under higher temperatures have faster rates of extension (Woodward *et al.*, 1986; Körner and Woodward, 1987; Morecroft *et al.*, 1992) and higher temperatures can also increase cell elongation rates (Prasad *et al.*, 2008). Gray *et al.* (1998) reported auxin induced cell expansion in *A. thaliana* under elevated temperature. While higher temperatures under natural conditions are often accompanied by water stress, the plants in the present cabinet based study were well watered. This limits the possibility of drought induced restriction of cell expansion (Yoo *et al.*, 2010) which often accompanies elevated temperature. Therefore, under high temperature conditions stomata would be widely spaced due to the expansion of epidermal cells leading to the observed decrease in SD.

In the present experiment involving increased temperature, ecotypes that showed the highest positive response (Rsch-4) and the highest negative response (Mt-0) in abaxial and adaxial SD were amongst the five ecotypes showing the highest value for SD for both leaf surfaces under ambient conditions. This supports the trend observed with elevated CO_2, ecotypes with a higher SD may also be more responsive to elevated temperature

In the present study, the overall increasing trend of SD with increasing altitude, which also represents a decreasing temperature gradient, is consistent with the decreases of SD observed in response to elevated temperature at all altitudes of origin. This decrease of SD at increased temperature (i.e. higher SD at the lower temperature) occurred at the same CO_2 concentrations maintained in the growth cabinets. Therefore, the results of the present study show that the stomatal density of *A. thaliana* has a direct response to temperature, independently of CO_2.

The study found that ecotypes from higher altitudes were in general the most responsive in their SD to higher temperature treatment. Lenoir *et al.* (2008) showed that plants most sensitive and vulnerable to global warming and distributional change were mountainous plants.

Factor analysis for elevated CO_2 treatment showed that all the measured variables loaded onto three factors. The clustering suggests that despite showing a diverse climatic background, ecotypes cluster together based on the response of their stomatal characters. However, it should be noted that some clusters could be explained based on a common altitudinal, geographical and climatic background.

In the response dendrogram, Col-0 diverged early on from the other ecotypes. Col-0 was the most responsive ecotype as it showed a significant response ($p<0.05$) for 14 out of the 15 variables under elevated CO_2 treatment. This high degree of responsiveness would have led to this ecotype clustering away from the others. It is interesting to note that Col-0 which is one of the most commonly used *A. thaliana* ecotypes in research was also one of the most responsive amongst the ecotypes in the present study. In comparison with the other ecotypes in the present study it also suggests that elevated CO_2 research utilizing this ecotype may not always provide a general depiction of the response of all *A. thaliana* ecotypes.

Factor analysis for elevated temperature showed that all the measured variables loaded strongly onto two or three factors. The SI was responsible for a lesser amount of the variation seen, especially in the ambient temperature factor analysis. This suggests that less of the variation amongst the temperature treatments was due to changes in the initial stomatal development step.

The *A. thaliana* ecotypes in the present study originated from different geographical regions and would have been subjected to diverse selection pressures in terms of temperature and CO_2 level. Thus, the ecotypes would have been adapted to their local microclimates (McKay *et al.*, 2001). While common geography and habitat explains some of the clustering, it cannot be regarded as the basis of all the clusters. Similarities amongst the stomatal parameters and the degree of responsiveness of each ecotype to elevated temperature led to a majority of the clustering patterns observed. The wide range of growth habitats of ecotypes suggests that they would have the plasticity in their physiological responses as well to adapt to temperature changes (Hoffman, 2002). Overall, the clustering did not show an altitudinal trend as the clusters included ecotypes from diverse altitudinal origins.

In the *Camellia sinensis* experiment the SD and SI decreased from the tip of the leaf to the base and this was seen under both CO_2 treatments. This typical trend has been reported in previous studies under ambient CO_2 conditions (Salisbury, 1927; Ferris *et al.*, 1996; Luomala *et al.*, 2005). Most studies use the mid region between the mid vein and the margin and the apex and base of the leaf for stomatal analysis (Reddy *et al.*, 1998; Apple *et al.*, 2000; Lin *et al.*, 2001; Tognetti *et al.*, 2001). The results of the present study showed that using the middle area of the leaf to be valid for *C. sinensis* plants as it showed an intermediate value in comparison to the extreme values of the tip and base. Thus, for further studies on stomatal characters of *C. sinensis*, using the mid area of the leaf would be a valid choice and this was used for the studies conducted in Sri Lanka.

Results of the present study indicated that no changes occur in the stomatal numbers of *C. sinensis* plants under elevated [CO_2]. The inverse relationship between stomatal numbers and [CO_2] initially reported by Woodward (1987) is not a universal trend exhibited by all plant species. Similar to the results seen here, a number of earlier studies have reported the lack of a stomatal frequency response to elevated [CO_2] in a range of plant species (Radoglou and Jarvis 1990b; Ryle and Stanley, 1992; Estiarte *et al.*, 1994; Case *et al.*, 1998; Poole *et al.*, 2000; Reid *et al*, 2003).

Although stomatal numbers did not change, stomatal conductance and transpiration decreased by about 25% under elevated [CO_2], which was probably brought about by a reduced stomatal aperture in the response of *C. sinensis* to elevated [CO_2]. Stomatal aperture level control was indicated in a number of other studies that did not show a stomatal frequency response to high [CO_2] (*Tradescantia* sp., Boetsch *et al.*, 1996; *Alnus glutinosa*, Poole *et al.*, 2000; Reid *et al.*, 2003; Ainsworth and Rogers, 2007; *C. sinensis,* Wijeratne *et al.*, 2007b).

It is also possible that the period of CO_2 enrichment (09 months) was insufficient to induce a stomatal frequency response (Miller-Rushing *et al.*, 2009), especially from a perennial species such as *C. sinensis*. Earlier studies have suggested that plants may lack the plasticity to significantly alter SD under high [CO_2] in a single generation/growing season (Malone *et al.*, 1993).

The elevated CO_2 treatment increased the leaf area of *C. sinensis*. Leaf area is an important component having a bearing on the physiological processes controlling total plant productivity and it varies with environmental conditions (Taylor *et al.*, 1994). A large body of experimental evidence supports the enhancement of leaf area during plant growth under elevated CO_2 (O'Leary and Knecht, 1981; Ceulemans *et al.*, 1995; Van der Kooij and De Kok, 1996; Atkinson *et al.*, 1997; Reddy *et al.*, 1998; Centritto *et al.*, 1999a; Zhao *et al.*, 2003).

The LMA value obtained for *C. sinensis* was within the range reported for tree species (50-200 gm^{-2}, Poorter *et al.*, 2009). An increase in LMA ($p=0.054$) was seen under elevated CO_2. A similar non significant increase in LMA was reported by Moutinho-Pereira *et al.* (2009) for *Vitis* sp while a significant increase in LMA is well documented in other species (Sasek and Strain, 1988; Sage *et al.*, 1989; Arp, 1991; Reddy *et al.*, 1998; Sims *et al.*, 1998; Roderick *et al.*, 1999; Ishizaki *et al.*, 2003; Poorter *et al.*, 2009). Previous studies have attributed this increase to the accumulation of non structural carbohydrates (TNC: starch, soluble sugars, fructans) due to imbalances in the source-sink relationship under elevated CO_2 (Reddy *et al.*, 1998; Sims *et al.*, 1998; Poorter *et al.*, 2009). Crop species are generally expected to be less prone to accumulation of non structural carbohydrates under elevated CO_2 (Pritchard *et al.*, 1999). However tea is regarded as a sink limited crop and this may contribute to the increase in LMA.

The elevated CO_2 treatment increased the net photosynthetic rate of *C. sinensis*. A similar response was observed by Wijeratne *et al.* (2007b) in a field experiment using mature *C. sinensis* plants grown under a [CO_2] of 600ppm. Furthermore, the observed 57% increase in net photosynthetic rate of the present study agrees well with the mean increase of 58% calculated by Drake *et al.* (1997) for a wide range of crop species across 60 experiments. It is also in agreement with the 53% increase in average photosynthesis reported by Curtis and Wang (1998) from a meta-analysis on woody plants. However, it should be noted that the field study by Wijeratne *et al.* (2007b) showed an increase of only 19% in comparison to the 57% reported herein. This may be related to the higher [CO_2] used in the present study and the inherent differences between cabinet based studies using small seedlings and open top chamber studies using mature tea bushes. In the latter type of CO_2 enrichment there is greater variation in the [CO_2] and the field plants would also be under increased environmental perturbations relative to plants growing under the uniform conditions of a controlled environmental cabinet study.

The reduction in g_s of 25% seen under elevated [CO_2] is in agreement with decreases in the range of 22%-28% reported in earlier studies of other species (Wullschleger *et al.*, 2002; Herrick *et al.*, 2004; Ainsworth and Rogers, 2007). It is also consistent with a 21% reduction in g_s seen in 13 long term field based studies on woody species (Medlyn *et al.*, 2001). The decrease in g_s in the present study was due to aperture level control as there were no parallel changes in SD. It is accepted that the aperture has the greatest control over the g_s with SD being only of secondary importance (Lawson, 2009) and numerous studies have proven this (Boetsch *et al.*, 1996; Poole *et al.*, 2000; Reid *et al.*, 2003; Ainsworth and Rogers, 2007; Wijeratne *et al.*, 2007b). Furthermore, the reported decrease in g_s is one of the most consistent responses of plants to growth under elevated [CO_2] (Long *et al.*, 2004).

The increase in net photosynthesis rate coupled with the lowered transpiration rate led to an increase in instantaneous water use efficiency. This is a commonly reported observation under elevated CO_2 conditions (Allen *et al.*, 2003). However, this property may not necessarily lead to drought tolerance of *C. sinensis* plants under future climate change conditions where droughts of increased intensity and length are predicted (Rosenzweig *et al.*, 2001). Other factors that determine the evaporative demand and supply such as leaf area, water transport in the stem and root systems may also play a part (Saxe *et al.*, 1998).

The elevated temperature treatment led to significant decreases in SD at the base of the *C. sinensis* leaf. When averaged across the different leaf positions, SD showed only a 2% increase in response to temperature elevation. A decrease in SD may lead to a decrease in water loss (Ciha and Brun, 1975; Beerling and Chaloner, 1993; Luomala *et al.*, 2005) and this may enable the tea plant to better adapt to potential conditions of high temperatures and water stress under climate change.

While relatively few elevated temperature studies have calculated SI, these have reported it to be both responsive (Ciha and Brun, 1975; Ferris *et al.*, 1996) and non responsive (Reddy *et al.*, 1998; Kouwenberg *et al.*, 2007) to this environmental parameter. The present study showed that the SI of the leaf tip and mid area increased significantly under elevated temperature.

The tea plants under elevated temperature conditions showed an increase in individual leaf area, which was not significant. Earlier studies have also noted an increase in leaf area with increasing temperature (*Dactylis glomerata* and *Sesleria albicans*, Woodward, 1979; *Glycine max*, Ciha and Brun, 1975; *Abutilon theophrasti* and *Amaranthus retroflexus,* Ackerly *et al.*, 1992; *Alchemilla alpina*, Morecroft and Woodward, 1996; Stirling *et al.*, 1998). Increasing temperature can stimulate cell division and cell elongation rates (Prasad *et al.*, 2008) and this would be a probable reason for an increase in leaf area under elevated temperature treatment. Studies have also found that plants under higher temperatures show faster rates of leaf extension (Körner and Woodward, 1987; Morecroft *et al.*, 1992; Morecroft and Woodward, 1996). Increased leaf area, while increasing photosynthetic capacity also provides a larger surface for evaporative cooling and thereby

The LMA values obtained under both treatments were within the range of 50-200 gm^{-2} reported for tree species by Poorter *et al.* (2009). They also reported that LMA decreases under elevated temperature, which was observed in the present study. LMA is used as a proxy for leaf thickness and a decrease in leaf thickness with increasing temperature has been previously reported particularly in altitude based studies. For instance, Kogami *et al.*, (2001) reported that the leaf was 1.5 times thinner at $26.8^{0}C$ in comparison to $13.5^{0}C$ in *Polygonum cuspidatum*. A decrease in LMA leads to increased vulnerability to herbivory and increased wear and tear of the leaf (Westoby *et al.*, 2002). Both these factors may affect commercial scale tea cultivation under future increases in temperature as the harvested crop is the young leaf itself. Poorter *et al.* (2009) reported that tropical species were more responsive in changing their LMA to a given change in temperature in comparison to boreal species. Thus, tea, a species originating from the tropics may have shown greater responsiveness in its LMA to increasing temperatures.

6. Conclusions

Based on the observations of this study, the following conclusions can be made on selected Sri Lankan tea cultivars:

(a) A majority of tea cultivars (mature tea bushes showed a reduction in SD with increasing altitude;

(b) The elevation experiment which was a reciprocal altitude experiment also showed that cultivars originating from the lower altitude had a higher SD and SI as did the higher altitude cultivars when they were grown at the lower altitude.

(c) Estate cultivars originating from the higher altitudes had a smaller number of stomata;

(d) The highest stomatal conductance of was shown by the cultivars growing at the lowest altitude irrespective of the altitude of origin of the propagatory material (where the mother plants were grown);

(e) Plants originating from the lower altitude had a significantly higher stomatal conductance than those from the higher altitude irrespective of which altitude they were grown in, which suggests a genetic basis for the higher stomatal conductance at the low altitude;

(f) A specific cultivar based stomatal response to environmental change was seen as some modified their stomatal numbers at the developmental step itself (TRI 2025, TRI 3019, TRI 3055, TRI 4042, CY9 and KEN 16/3);

(g) The study identified tea cultivars that showed a high degree of responsiveness to environmental change in terms of all stomatal variables (TRI 3014 and TRI 4053) and those that showed a low overall response (TRI 2043);

(h) Cultivars had a significantly higher net assimilation rate (NAR) when grown at the low altitude. Thus they were more effective in their biomass production in terms of amount of photosynthetic tissue;

(i) Cultivars that were grown at the lower altitude had a higher root to shoot ratio;

(j) Cultivars originating from the lower altitude showed a higher leaf area than those from the higher altitude;

(k) Tea cultivars showed an increase in leaf thickness with increasing altitude;

(l) The specific leaf area (SLA) was lower at the high altitude. As a lower SLA is indicative of thicker leaves this further confirms that leaf thickness increases with increasing altitude;

(m) Leaves sampled from the lowest altitude (Ratnapura) showed the highest PSR and palisade to total leaf ratios and the lowest spongy parenchyma to total leaf thickness ratio. The leaves at the mid altitude (Hantane) had the highest spongy parenchyma to total leaf thickness ratio;

(n) Estate cultivars from each altitude consistently showed a lower PSR and palisade to total leaf thickness ratio;

(o) Cultivars decreased their Relative Water Content (RWC) under water stress and TRI 3014 and CY9 gave an indication that they were relatively tolerant of water stress;

(p) TRI 2026 was water stress susceptible cultivar that showed high leaf fall under stress;

(q) A majority of the variance amongst the cultivars across an altitudinal gradient could be explained by the leaf anatomical allometry and stomatal-density related characters;

(r) The cluster analysis showed that the estate cultivars sampled in the study were more responsive to their growth environment in comparison to the newly developed TRI cultivars.

UK based studies

Based on the observations of this study, the following conclusions can be made with regard to elevated CO_2 and *A. thaliana* ecotypes:

(a) Ecotypic variation exists in the response to elevated CO_2 of SD, ED and ED of both leaf surfaces of *A. thaliana* ecotypes. A majority of ecotypes increase their SD and ED of both leaf surfaces when growth CO_2 concentration is increased from 400ppm to 800ppm. This shows that the size of epidermal cells decrease under elevated CO_2;

(b) The SD of *A. thaliana* ecotypes showed an overall increasing trend with increasing altitude of origin though this was not consistent;

(c) *A. thaliana* ecotypes show a differential SD response to the natural CO_2 gradient experienced with decreasing altitude (a decrease in SD) and to the artificial increase in CO_2 in the growth chambers (an increase in SD);

(d) The response of stomatal development of *A. thaliana* ecotypes to CO_2 (i.e. stomatal index) varied based on the leaf surface with the abaxial surface being relatively more responsive;

(e) Factor analysis showed that a majority of the variation in the ecotypes under both ambient and elevated CO_2 treatments were due to SD and ED;

(f) Clustering patterns were explained mostly by the climate and degree of responsiveness in stomatal characters rather than the altitudinal origin of ecotypes.

Based on the observations of this study, the following conclusions can be made with regard to elevated temperature and *A. thaliana* ecotypes:

(g) Ecotypic variation exists in the response to elevated temperature of SD, ED, SI, GCL and PCI of both leaf surfaces of *A. thaliana* ecotypes. A majority of ecotypes decrease their SD and ED of both leaf surfaces when growth temperature is increased from 21° to $28^{\circ}C$. This decrease in SD was due to the concurrent decrease in ED caused by expansion of epidermal cells;

(h) *A. thaliana* ecotypes with a higher initial SD were more responsive to elevated temperature. Ecotypes from higher altitudes were also in general the most responsive in their SD and ED to elevated temperature;

(i) The response of stomatal development of *A. thaliana* ecotypes to elevated temperature (i.e. stomatal index) varied based on the leaf surface with the adaxial surface being relatively more responsive;

(j) The SD of *A. thaliana* ecotypes showed a overall increasing trend with increasing altitude of origin though this was not consistent;

(k) Factor analysis showed that a majority of the variation in the ecotypes under both ambient and elevated temperature treatments were due to SD and ED;

(l) Clustering patterns were explained mostly by the climate and degree of responsiveness in stomatal characters rather than the altitudinal origin of ecotypes.

Based on the observations of this study, the following conclusions can be made on the response of *C. sinensis* to elevated CO_2:

(a) The stomatal frequency of *C. sinensis* was insensitive to an increase in CO_2 concentration from 400ppm to 800ppm;

(b) A decrease in stomatal conductance indicated that *C. sinensis* shows an aperture level response to elevated CO_2;

(c) Elevated CO_2 increased the net photosynthetic rate of *C. sinensis*. The increases observed in leaf area and leaf mass per unit area (LMA) would have contributed to this response;

(d) The increase in photosynthesis and decrease in stomatal conductance under elevated CO_2 led to an increase in the instantaneous water use efficiency of *C. sinensis*;

Based on the observations of this study, the following conclusions can be made on the response of *C. sinensis* to elevated temperature:

(a) A temperature increase from 21° to 31°C stimulates the seedling growth of *C. sinensis*;

(b) Increases in stomatal density and stomatal index combine to increase leaf gas exchange capacity of *C. sinensis* with increasing temperature;

(c) Increased temperature decrease leaf mass per unit area (LMA) of *C. sinensis*, which however did not decrease leaf photosynthetic capacity because of associated increases leaf gas exchange capacity and leaf area;

(d) Temperature induced changes in leaf gas exchange capacity of *C. sinensis* are caused by both increased cell expansion rates and increased initiation of stomata;

7. References:

- Ackerly, D.D., Coleman, J.S., Morse, S.R., & Bazzaz. F.A. (1992). CO_2 and temperature effects on leaf area production in two annual plant species. *Ecology, 73*, 1260-1269.
- Ainsworth, E.A. & Rogers, A. (2007). The response of photosynthesis and stomatal conductance to rising [CO_2]: mechanisms and environmental interactions. *Plant, Cell and Environment, 30*, 258-270.
- Allen, L.H., Pan, D., Boote, K.J., Pickering, N.B., & Jones, J.W. (2003). Carbon dioxide and temperature effects on evapotranspiration and water use efficiency of Soybean. *Agronomy Journal, 95*, 1071-1081.
- Anonymous. (2002). The suitability of tea clones for the different regions'. TRI Advisory Circular, Serial number 6/02. The Tea Research Institute of Sri Lanka
- Apple, M.E., Olszyk, D. M., Ormrod, D.P., Lewis, J., Southworth, D. & Tingey, D.T. (2000). Morphology and stomatal function of Douglas Fir needles exposed to climate change: elevated CO_2 and temperature. *International Journal of Plant Sciences, 161*, 127-132.
- Arp, W.J. (1991). Effects of source-sink relations on photosynthetic acclimation to elevated CO_2. *Plant, Cell & Environment, 14*, 869–875.
- Ashton, P.M.S. & Berlyn, G.P. (1992). Leaf adaptations of some *Shorea* species to sun and shade. *New Phytologist, 121,* 587-596.
- Atkinson, C.J., Taylor, J.M., Wilkins, D. & Besford, R.T. (1997). Effects of elevated CO_2 on chloroplast components, gas exchange and growth of oak and cherry. *Tree Physiology, 17*, 319-325.
- Balasuriya, J. (2000). Effect of elevation on net total dry matter production and yield of two clones of tea in Sri Lanka. *Sri Lanka Journal of Tea Science, 66*, 49-68.

- Beerling, D.J. & Chaloner, W.G. (1993). The impact of atmospheric CO_2 and temperature change on stomatal density: observations from *Quercus robur* lammas leaves. *Annals of Botany, 71*, 231-235.
- Boetsch, J, Chin, J, Ling, M. & Croxdale, J. (1996). Elevated carbon dioxide affects the patterning of subsidiary cells in *Tradescantia* stomatal complexes. *Journal of Experimental Botany, 47*, 925-931.
- Bresson, C.C., Vitasse, Y., Kremer, A., & Delzon, S. (2011). To what extent is altitudinal variation of functional traits driven by genetic adaptation in European oak and beech? *Tree Physiology, 31*, 1164-1174.
- Case, A.L., Curtis, P.S. & Snow, A.A. (1998). Heritable variation in stomatal response to elevated CO_2 in wild radish, *Raphanus raphanistrum* (Brassicaceae). *American Journal of Botany, 85 (2),* 253-258.
- Casson, S. & Gray, J.E. (2008). Influence of environmental factors on stomatal development. *New Phytologist, 178*, 9-23.
- Centritto, M., Lee, H.S.J., & Jarvis, P.G. (1999a). Interactive effects of elevated [CO_2] and drought on cherry (*Prunus avium*) seedlings: growth, whole plant water use efficiency and water loss. *New Phytologist, 141*, 129-140.
- Ceulemans, R., Praet L. & Jiang, X. N. (1995). Effects of CO_2 enrichment, leaf position and clone on stomatal index and epidermal cell density in Poplar (*Populus*). *New Phytologist, 131(1),* 99-107.
- Ciha, A.J. & Brun, W.A. (1975). Stomatal size and frequency in soybeans. *Crop Science, 15,* 309-313.
- Clifford, S.C., Black, C.R., Roberts, J.A., Stronach, M., Singleton-Jones, P.R., Mohamed, A.D., & Azam-Ali, S.N. (1995). The effect of elevated atmospheric CO_2 and drought on stomatal frequency in groundnut (*Arachis hypogaea* (L.)). *Journal of Experimental Botany, 46,* 847-852.
- Cordell, S., Goldstein, G., Meinzer, F. C., & Handley, L. L. (1999). Allocation of nitrogen and carbon in leaves of metrosideros polymorpha regulates carboxylation capacity and $\delta^{13}C$ along an altitudinal gradient. *Functional Ecology, 13*, 811-818.

- Damayanthi, M.M.N., Mohotti, A.J. & Nissanka, S.P. (2010). Comparison of tolerant ability of mature field grown tea (*Camellia sinensis* L.) cultivars exposed to a drought stress in Passara area. *Tropical Agricultural Research, 22 (1),* 66 – 75.
- De Costa, W.A. J.M., Mohotti, A.J. & Wijeratne, M.A. (2007a). Ecophysiology of Tea. *Brazilian Journal of Plant Physiology, 19 (4),* 299-332.
- Drake, B.G., Gonzalez-Meler, M.A., & Long, S.P. (1997). More efficient plants: a consequence of rising atmospheric CO_2? *Annu. Rev. Plant. Physiol. Plant Mol. Biol.* 48, 609-639.
- Estiarte, M., Penuelas, J., Kimball, B.A., Idso, S.B., LaMorte, R.L., Pinter, P.J., Wall, G.W., & Garcia, R.L. (1994). Elevated CO_2 effects stomatal density of wheat and sour orange trees. *Journal of Experimental Botany, 45*, 1665-1668.
- Evans, J.R. & Poorter, H. (2001). Photosynthetic acclimation of plants to growth irradiance: the relative importance of SLA and nitrogen partitioning in maximising carbon gain. *Plant Cell & Environment, 24,* 755–768.
- Ferris, R., Nijs, I., Behaeghe, T. & Impens, I. (1996). Elevated CO_2 and temperature have different effects on the leaf anatomy of perennial ryegrass in spring and summer. *Annals of Botany, 78*, 489-476.
- Gale, J. (1972). Availability of carbon dioxide for photosynthesis at high altitudes: theoretical considerations. *Ecology, 53*, 494-497.
- Herrick, J.D., Maherali, H., & Thomas, R.B. (2004). Reduced stomatal conductance in sweetgum (*Liquidambar styraciflua*) sustained over long-term CO_2 enrichment. *New Phytologist, 162,* 387–396.
- Hetherington, A.M. & Woodward, F.I. (2003). The role of stomata in sensing and driving environmental change. *Nature, 424*, 901-908.
- Hoffman, M.H. (2002). Biogeography of *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae). *Journal of Biogeography, 29*, 125-134.
- Hossain, M.I., Khatun, A. et al. (2010). Effect of drought on physiology and yield contributing characters of sunflower. *Bangladesh J. Agril. Res.*, *35*(1), 113-124.
- IPCC (2007). Climate change 2007: The physical science basis. In: Soloman S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL, (Eds.) *Contribution of*

working group I to the fourth assessment report of the intergovernmental panel on climate change. UK. Cambridge University Press.

- Ishizaki, S., Hikosaka, K. & Hirose, T. (2003). Increase in leaf mass per area benefits plant growth at elevated CO_2 concentration. *Annals of Botany, 91*, 905-914.
- Jin, B., Wang, J., Jiang, K., Wang, Y., Jiang, C., Ni, C., Wang, Y., & Teng, N. (2011).The effect of experimental warming on leaf functional traits, leaf structure and leaf biochemistry in *Arabidopsis thaliana. BMC Plant Biology, 11,* 2-10.
- Kenzo, T., Ichie, T., Yoneda, R., Kitahashi, Y., Watanabe, Y., Ninomiya, I., & Koike, T. (2004). Interspecific variation of photosynthesis and leaf characteristics in canopy trees of five species of Dipterocarpaceae in a tropical rain forest. *Tree Physiology, 24,* 1187-1192.
- Kogami, H., Hanba, Y.T., Kibe, T., Terashima, I. & Masuzawa, T. (2001). CO_2 transfer conductance, leaf structure and carbon isotope composition of *Polygonum cuspidatum* leaves from low and high altitudes. *Plant Cell and Environment, 24*, 529-538.
- Körner, C. & Cochrane, P.M. (1985). Stomatal responses and water relations of Eucalyptus pauciflora in summer along an elevational gradient. *Oecologia, 66,* 443-455.
- Körner, C. & Diemer, M. (1987). *In situ* photosynthetic responses to light, temperature and carbon dioxide in herbaceous plants from low and high altitude. *Functional Ecology, 1,* 179-194.
- Körner, C., Bannister, P. & Mark, A.F. (1986). Altitudinal variation in stomatal conductance, nitrogen content and leaf anatomy in different plant life forms in New Zealand. *Oecologia, 69 (4),* 577-588.
- Kouwenberg, L.L.R., Kűrschner, W.M., & McELwain, J.C. (2007). Stomatal frequency change over altitudinal gradients: prospects for paleoaltimetry. *Reviews in Mineralogy and Geochemistry, 66*, 215-241.
- Lawlor, D. W. & Mitchell, R. A. C. (1991). The effects of increasing CO_2 on crop photosynthesis and productivity: a review of field studies. *Plant Cell and Environment, 14,* 807-818.
- Lawson, T. (2009). Guard cell photosynthesis and stomatal function. *New Phytologist, 181,* 13-34.

- Lawson, T., Craigon, J., Black, C.R., Colls, J.J., Landon, G., & Weyers, J.D.B. (2002). Impact of elevated CO_2 and epidermal characteristics in potato (*Solanum tuberosum* L.). *Journal of Experimental Botany, 53,* 737-746.
- Lenoir, J., Gégout, J.C., Marquet, P. A., de Ruffray, P., & Brisse, H. (2008). A significant upward shift in plant species optimum elevation during the 20th century. *Science 320,* 1768-1771.
- Long, S.P., Ainsworth, E.A., Rogers, A., & Ort, D.R. (2004). Rising atmospheric carbon dioxide: plants FACE the future. *Annual Review of Plant Biology 55,* 591-628.
- Luo, J, Zang, R., & Li, C. (2006). Physiological and morphological variations in *Picea asperata* populations originating from different altitudes in the mountains of southwestern China. *Forest Ecology and Management, 221,* 285-290.
- Luomala, E., Laitinen, K., Sutinen, S., Kellomaki, S., & Vapaavuori, E. (2005). Stomatal density, anatomy and nutrient concentrations of Scots pine needles are affected by elevated CO_2 and temperature. *Plant, Cell and Environment, 28,* 733-749.
- Malone, S.R., Mayeux, H.S., Johnson, H.B. & Polley, H.W. (1993). Stomatal density and aperture length in four plant species grown under subambient CO_2 gradient. *American Journal of Botany, 80(12), 141 3-1418.*
- McKay, J.K., Bishop, J.G., Lin, J., Richards, J.H., Sala, A., & Mitchell-Olds, T. (2001). Local adaptation across a climatic gradient despite small effective population size in the rare sapphire rockcress. *Proceedings of the Royal Society, London, 268,* 1715-1721.
- Medlyn B. E., Barton, C. V. M., Broadmeadow, M. S. J., Ceulemans, R., De Angelis, P., Forstreuter, M., Freeman, M., Jackson, S. B., Kellomäki, S., Laitat, E., Rey, A., Roberntz, P., Sigurdsson, B. D., Strassemeyer, J., Wang, K., Curtis, P. S. & Jarvis, P. G. (2001). Stomatal conductance of forest species after long-term exposure to elevated CO_2 concentration: a synthesis. *New Phytologist*, 149, 247–264.
- Miller-Rushing, A.J., Primack, R.B., Templer, P.H., Rathbone, S. & Mukunda, S. (2009). Long-term relationships among atmospheric CO_2, stomata and intrinsic water use efficiency in individual trees. *American Journal of Botany, 96 (10),* 1779-1786.
- Morecroft, M. & Woodward, F.I. *(*1990*)* Experimental investigations on the environmental determination of $\Delta^{13}C$ at different altitudes. *Journal of Experimental Botany, 41,* 1303-1308.

- Morecroft, M. & Woodward, F.I. (1996). Experiments on the cause of altitudinal differences in the leaf nutrient contents, size and $\delta^{13}C$ of *Alchemilla alpina. New Phytologist, 134,* 471-479.
- Morecroft, M., Woodward, F.I., & Marrs, R.H. (1992). Altitudinal trends in leaf nutrient contents, leaf size and $\delta^{13}C$ of *Alchemilla alpina. Functional Ecology, 6,* 730-740.
- Morison, J.I.L. (1998). Stomatal responses to increased CO_2 concentration. *Journal of Experimental Botany*, *49*, 443-452.
- Moutinho-Pereira, J., Goncalves, B., Bacelar, E., Boaventura Cunha, J., Coutinho, J., & Correia, C.M. (2009). Effects of elevated CO2 on grapevine (*Vitis vinifera* L.): Physiological and yield attributes. *Vitis, 48,* 159-165.
- Niinemets, U. (1999). Components of leaf dry mass per area- thickness and density – alter leaf photosynthetic capacity in reverse directions in woody plants. *New Phytologist, 144*, 35-47.
- O'Leary, J. & Knecht, G.N. (1981). Elevated CO_2 concentration increases stomate numbers in *Phaseolus vulgaris* leaves. *Botanical Gazette, 142(4),* 438-441.
- Peguero-Pina, J.J., Gil-Peregrin, E. & Morales, F. (2009). Photosystem II efficiency of the palisade and spongy mesophyll in Quercus coccifera using adaxial/ abaxial illumination and excitation light sources with wavelengths varying in penetration into the leaf tissue. *Photosynthetic Research, 99,* 49-61.
- Poole, I., Lawson, T., Weyers, J.D.B. & Raven, J.A. (2000). Effect of elevated CO_2 on the stomatal distribution and leaf physiology of *Alnus glutinosa. New Phytologist*, *145*, 511-521.
- Poorter, H., Niinemets, U., Poorter, L, Wright, I.J. & Villar, R. (2009). Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytologist, 182,* 565-588.
- Prasad, P.V.V., Staggenborg, S.A., & Ristic, Z. (2008). Impacts of Drought and/or Heat Stress on Physiological, Developmental, Growth, and Yield Processes of Crop Plants. Response of crops to limited water: Understanding and modeling water stress effects on plant growth processes. Advances in agricultural systems modeling series 1. 301-355. Madison, USA.
- Pritchard, S.G., Rogers, H.H., Prior, S.A., & Peterson, C.M. (1999). Elevated CO_2 and plant structure: a review. *Global Change Biology*, *5*, 807-837.

- Qiang, W., Wang, X., Chen, T., Feng, H., An,L., He, Y., & Wang, G. (2003). Variations of stomatal density and carbon isotope values of *Picea crassifolia* at different altitudes in the Qilian Mountains, *Trees, 17*, 258-262.
- Radoglou, K.M. & Jarvis P.G. (1990b). Effects of CO_2 enrichment on four poplar clones. II. Leaf surface properties. *Annals of Botany,* 65, 627-632.
- Raven, J.A., Evert, R.F., & Eichorn, S.E. (2005). *Biology of Plants.* 7th Edition. W.H.Freeman and Company, New York.
- Reddy, K.R., Robana, R.R., Hodges, H.F., Liu, X.J., & McKinion, J.M. (1998). Interactions of CO_2 enrichment and temperature on cotton growth and leaf characteristics. *Environmental and Experimental Botany, 39,* 117-129.
- Reid, C.H., Maherali, H., Johnson, H.B., Smith, S.D., Wullschleger, S.D. & Jackson, R.B. (2003). On the relationship between stomatal characters and atmospheric CO_2. *Geophysical Research Letters, 30 (19*), 1983.
- Richards, A.V. (1967). Some observations on the performance of the popular TRI and Estate clones. *The Tea Quarterly, 38 (3),* 245-248.
- Roderick, M.L. Berry, .L. & Noble, I.R. (1999). The relationship between leaf composition and morphology at elevated CO_2 concentrations. *New Phytologist, 143*, 63-72.
- Rosenzweig, C., Iglesius, A., Yang, X.B., Epstein, P.R., & Chivian, E. (2001). Climate change and extreme weather events – implications for food production, plant diseases and pests. *Global Change and Human Health*, 2, 90-104.
- Ryle, G.J.A. & Stanley, J. (1992). Effect of elevated CO_2 on stomatal size and distribution in perennial ryegrass. *Annals of Botany 69,* 563-565.
- Sage, R.F., Sharkey, T.D., & Seeman, J.R. (1989). Acclimation of photosynthesis to elevated CO_2 in five C_3 species. *Plant Physiology, 30*, 1086-1106
- Salekjalali, M., Haddad, R. et al. (2011). Analysis of antioxidant enzyme activity during reproductive stages of barley under drought stress. *Journal of Ecobiotechnology* , *3*(10), 40-47.
- Salisbury, E.J. (1927). On the causes and ecological significance of stomatal frequency, with special reference to the woodland flora. *Philosophical transactions of the Royal Society of London, B, Biological Sciences, 216*, 1-65.

- Sasek, T.W. & Strain, B.R. (1988). Effects of CO_2 enrichment on the growth and morphology of Kudzu (*Pueraria lobata*). *Weed Science, 36 (1),* 28-36.
- Saxe H., Ellsworth, D.S. & Heath, J. (1998). Tree and forest functioning in an enriched CO_2 atmosphere. *New Phytologist*, 139, 395-436.
- Schoettle, A.W., & Rochelle, S.G. (2000). Morphological variation of *Pinus flexilus* (Pinaceae), a bird-dispersed pine, across a range of elevations. *American Journal of Botany, 87 (12),* 1797-1806.
- Sims, D.A., Seeman, J.R., & Luo, Y. (1998). Elevated CO_2 has independent effects on expansion rates and thickness of soybean leaves across light and nitrogen gradients. *Journal of Experimental Botany, 140*, 343-354.
- Smith, W.K., Vogelmann, T.C., Delucia, E.H., Bell, D.T., & Shepherd, K.A. (1997). Leaf form and photosynthesis. *BioScience, 47,* 785-793.
- Stenstrom, A., Jonsdottir, I. M., & Augner, M. (2002). Genetic and environmental effects on morphology in clonal sedges in the eurasian arctic. *American Journal of Botany, 89(9),* 1410–1421.
- Stirling, C.M., Heddell-Cowie, M., Jones, M.L., Ashenden, T.W., & Sparks, T.H. (1998). Effects of elevated CO_2 and temperature on growth and allometry of five native fast-growing annual species. *New Phytologist, 150,* 665-674.
- Taylor, G., Ranasinghe, S., Bosac, C., Gardner, S.D.L. & Ferris, R. (1994). Elevated CO_2 and plant growth: celluar mechanisms and responses of whole plants. *Journal of Experimental Botany, 45,* Special Issue, 1761-74.
- Teng, N., Wang, J., Chen, T., Wu, X., Wang, Y., & Lin, J. (2006). Elevated CO_2 induces physiological, biochemical and structural changes in leaves of *Arabidopsis thaliana. New Phytologist, 172*, 92-103.
- Terashima, I. & Saeki, T. (1983). Light environment within a leaf I. Optical properties of paradermal sections of Camellia leaves with special reference to differences in the optical properties of palisade and spongy tissues. *Plant and Cell Physiology, 24,* 1493-1501.
- Tognetti, R., Sebastiani, L., Vitagliano, C., Raschi, A. & Minnocci, A. (2001). Responses of two olive tree (*Olea europaea* L.) cultivars to elevated CO_2 concentration in the field. *Photosynthetica, 39(3),* 403-410.

- Van der Kooij, T.A.W & De Kok, L.J. (1996). Impact of elevated CO_2 on growth and development of *Arabidopsis thaliana* L. *Phyton, 36,* 173-184.
- Velázquez-Rosas, N., Meave, J., & Vázquez-Santana, S. (2002). Elevational variation of leaf traits in mountain rain forest tree species at La Chinantla, southern Mexico. *Biotropica, 34,* 534-546.
- Vidhana Arachchi, L.P., Vidhana Arachchi, V.R.M. et al. (2012). Effect of soil physical and water stress on biochemical aspects of Coconut seedlings (*Cocos nucifera* L.). *The Journal of Agricultural Sciences, 7,* 3.
- Vitousek, P.M., Field, C.B. & Matson, P.A. (1990). Variation in foliar $\delta^{13}C$ in Hawaiian *Metrosideros polymorpha*: A case of internal resistance? *Oecologia, 84 (3),* 362-370.
- Ward, J.K. & Strain, B.R. (1997). Effects of low and elevated CO_2 partial pressure on growth and reproduction of *Arabidopsis thaliana* from different elevations. *Plant, Cell and Environment 20,* 254-260.
- Westoby, M., Falster, D.S., Moles, A.T., Vesk, P.A., & Wright, I.J. (2002). Plant ecological strategies: some leading dimensions of variation between species. *Annual Review of Ecological Systems, 33,* 125-159.
- Wickramaratne, M.R.T. (1981). Genotype-environment interactions in tea (*Camellia sinensis* L.) and their implications in tea breeding and selection. *The Journal of Agricultural Science, 96,* 471-478.
- Wijeratne, M.A., Anandacoomaraswamy, A., Amarathunga, M.K.S.L.D., Ratnasiri, J., Basnayake, B.R.S.B., & Kalra, N. (2007a). Assessment of impact of climate change on productivity of tea (*Camellia sinensis* L.) plantations in Sri Lanka. *Journal of the National Science Foundation 35(2),* 119-126.
- Wijeratne, M.A., Ratnasiri, J., & Premathunga, E.W.T.I. (2007b). Effect of CO_2 fertilization on growth and yield of mature tea in the low country wet zone of Sri Lanka. *Journal of Plantation Crops, 35(1),* 56-58.
- Wijeratne, T.L., Mohotti, A.J. & Nissanka, S.P. (2008). Impact of long term shade on physiological, anatomical and biochemical changes in tea (*Camellia sinensis* L.(O) Kuntze. *Tropical Agricultural Research 20,* 376 – 387.
- Woodward, F.I. (1979). The differential temperature responses of the growth of certain plants species from different altitudes. I. Growth analysis of *Phleum alpinum* L., *P.*

bertolonii D.C., *Sesleria albicans* Kit. and *Dactylis glomerata* L. *New Phytologist, 82*, 385-395.

- Woodward, F.I. (1983). The significance of interspecific differences in specific leaf area to the growth of selected herbaceous species from different altitudes. *New Phytologist, 95*, 313-323.
- Woodward, F.I. (1986). Ecophysiological studies on the shrub *Vaccinium myrtillus* L. taken from a wide altitudinal range. *Oecologia, 70*, 580-586.
- Woodward, F.I. (1987). Stomatal numbers are sensitive to increases in CO_2 from pre-industrial times. *Nature, 327*, 617-618.
- Woodward, F.I., & Kelly, C.K. (1995). The influence of CO_2 concentration on stomatal density. *New Phytologist 131*, 311-327.
- Woodward, F.I., Lake, J.A. & Quick, W.P. (2002). Stomatal development and CO_2: ecological consequences. *New Phytologist, 153*, 477-484.
- Xie, S., Sun, B., Yan, D., & Du, B. (2009). Altitudinal variation in *Ginkgo* leaf characters: Clues to paleoelevation reconstruction. *Science in China Series D: Earth Sciences, 52*, 2040-2046.
- Yoo, C.Y., Pence, H.E., Jin, J.B., Miura, K., Gosney, M.J., Hasegawa, P.M., & Mickelbart, M.V. (2010) The *Arabidopsis* GTL1 transcription factor regulates water use efficiency and drought tolerance by modulating stomatal density via transrepression of SDD1. *Plant Cell, 22*, 4128-4141.
- Zhao, D, Reddy, K.R., Kakani, V.G., Read, J.J., & Sullivan, J.H. (2003). Growth and physiological responses of cotton (*Gossypium hirsutum* L.) to elevated CO_2 and ultra-violet-B radiation under controlled environmental conditions. *Plant Cell and Environment, 26*, 771-782.

8. Problems if any encountered during the implementation of the project

A problem encountered was the non availability of a working photosynthetic meter for analysis of photosynthesis. Thus, other allometric ratios such as palisade to spongy layer ratio and Net Assimilation rate was used as indicators of this physiological parameter.

9. Major findings and follow up activities

Future increases in CO_2 concentration and temperature under predicted climate change are likely to impact upon plant growth and function. While stomata play a key role in a plant's response due to its central role in the gas exchange pathway and thus in the growth and function of a plant there is limited knowledge on the response of stomata to these variables. This is especially true in the context of woody crop species. Furthermore, the response of stomatal characters across an altitudinal gradient is of interest due to the natural gradient that would exist in CO_2 concentration [CO_2] and temperature. To this end, the present study concentrated on ecotypes of *A. thaliana* originating from an altitudinal gradient as well as on *C. sinensis*, a species of economic interest to Sri Lanka which also grows across an altitudinal gradient under field conditions.

The study found that *A. thaliana* ecotypes showed diverse ecotype based responses in their stomatal density (SD), epidermal density (ED) and stomatal index (SI) to both elevated CO_2 and temperature. A novel outcome of this study was the observation that *A. thaliana* ecotypes with a higher initial SD were more responsive to elevated temperature. While earlier studies have shown a species based response to these variables, the present study shows that both the magnitude and direction of response of stomatal dimensions and frequency can vary amongst ecotypes within a species. The study also showed that a majority of ecotypes have the phenotypic plasticity to adapt to future changes in CO_2 concentration. This may have an impact on the natural *A. thaliana* populations as studies have shown that changes in CO_2 levels have the potential to alter the genetic composition of plant species over evolutionary time scales (Ward and Strain, 1997).

It should be noted that though altitude had an effect on the response shown, a majority of stomatal characters did not a show a clear linear response to altitude. The variations in stomatal characters observed here were presumably partly caused by the diverse habitats from which the ecotypes originate as shown by earlier studies using different plant species (*Picea asperata,* Luo *et al*., 2006; *Gingko biloba,* Xie *et al*., 2009).

The SD of *C. sinensis* did not vary in response to elevated CO_2 treatment. Conversely, in terms of the response of *C. sinensis* to elevated temperature it was seen that stomatal numbers were more relatively more responsive. Elevated CO_2 increased the net photosynthetic rate of *C. sinensis*. The increases observed in leaf area and leaf thickness probably contributed to this response.

A 10°C increase in temperature from 21°C to 31°C stimulated growth of *C. sinensis* seedlings and overall led to positive effects in terms of their total capacity for photosynthesis and gas exchange. The increases in SD and SI may combine to increase leaf gas exchange capacity of *C. sinensis* with increasing temperature while an increase in leaf area is conducive to a higher photosynthetic rate per plant (De Costa, 2004). This suggests that a 10°C temperature increase would be beneficial for *C. sinensis* and is in agreement with an earlier study based on a crop model for Sri Lankan tea (Wijeratne *et al*., 2007a). It should be noted that this would be valid only for tea that is at present growing under a non-stressful temperature of around 21°C. The study by Wijeratne *et al.* (2007a) showed that tea presently growing at a higher temperature (~28°C), which was already in the upper threshold may be adversely affected by further temperature increases. The present study showed that the temperature increase led to a decrease in WUE, which may be a concern for a rain fed crop such as tea under predicted water limited scenarios (Rosenzweig *et al*., 2001; IPCC, 2007).

The similarities observed between the present cabinet based elevated CO_2 study on *C. sinensis* and the open top chamber field study by Wijeratne *et al.* (2007b) validates the use of the present study to make predictions on field grown tea in Sri Lanka. However, productivity of crops under field conditions is affected by factors such as temperature, humidity, water supply, solar radiation, nutrient supply and these may interact with the effects of CO_2 enrichment (Lawlor and

Mitchell, 1991). In fact, a study by Wijeratne *et al.* (2007a) revealed that while CO_2 enrichment would increase productivity of the tea plant, this effect may be reduced due to rising atmospheric temperatures and drier weather conditions. Future increases in both CO_2 and temperature may bring mixed results on the WUE of *C. sinensis* necessitating further research on the interaction of these two variables. Possible adaptive measures to counteract this identified threat would be to encourage the planting of drought tolerant tea cultivars and soil moisture conservation in marginal areas (Wijeratne *et al.*, 2007a).

A cultivar based response to altitude was shown by the *C. sinensis* cultivars in the field based study where some cultivars modified their stomatal numbers at the developmental step itself (TRI 2025, TRI 3019, TRI 3055, TRI 4042, CY9 and KEN 16/3).

Anatomical studies showed that leaf thickness increased with increasing altitude in agreement with earlier altitude based studies. The highest palisade:spongy parenchyma ratio (PSR) was shown by the cultivars sampled at the lowest altitude while the estate cultivars showed the lowest PSR at each altitude. A greater PSR is related to a high potential for faster gas exchange (Sack and Frole, 2006). Therefore, the former result may be due to the availability of resources conducive to a high photosynthetic rate at the lower altitude. Factor analysis showed that a majority of the variance amongst the cultivars across an altitudinal gradient was explained by the leaf anatomical allometry and stomatal density-related characters. In the cluster analysis it was seen that estate cultivars sampled in the study were more responsive to environmental change in comparison to the newly developed TRI cultivars.

Tea cultivars that are specially adapted for a specific location are necessary in the absence of 'universal varieties' that perform well under all conditions (Wickramaratne, 1981). The present study looked at the manner in which a cultivar's stomatal and anatomical characters respond to diverse conditions experienced at different altitudes. These characters have a direct bearing on a plant's photosynthetic capacity and hence its ultimate productivity. Therefore, the highly or less responsive cultivars identified herein could form a basis for the selection of parent material for future breeding trials. The identification of cultivars responsive to their environment would also be important when breeding crops which would grow under future changes in the climate. Those that are able to change the number or the dimensions of the stomata would be better equipped to adapt under future changes in CO_2, temperature and precipitation. Plasticity in leaf anatomical characters would also allow cultivars to adjust their photosynthetic ability according to the environmental conditions.

A majority of the stomatal and anatomical traits reported here have not been studied previously and serves to fill a lacuna in information on popular tea cultivars. These characters could also now be included for consideration when developing new cultivars in the future.

These findings complement the existing knowledge on species-based responses to elevated CO_2 and temperature. Studies on crop species such as *C. sinensis* are important as agricultural productivity could be affected by climate change and understanding potential effects prior to those changes would allow time for adjustment (Reddy *et al.*, 1998). The ecotype based study on *A. thaliana* clearly shows the intra-specific variability present in this model plant and the importance of avoiding generalisations based on a few ecotypes.

Potential future research

The study outlined above provides the basis for further investigation in a number of areas. Stomatal characters across a larger altitudinal gradient could be studied which would be useful in observing trends in various parameters with changing altitude. Future field based studies could incorporate a larger number of tea cultivars sampled from the main tea growing regions in Sri Lanka. This would add to the knowledge base of stomatal and leaf anatomical characters of Sri Lankan tea cultivars initiated through the present study. Multivariate studies that incorporate physiological measurements along with the yield and other parameters measured herein would provide a deeper understanding of the cultivar response to the environment. Also, further studies related to soil water deficit would lead to a better understanding in choosing cultivars with better water use efficiency under future climate conditions.

The growth chamber based studies on *C. sinensis* provided insights into the possible response of tea to elevated CO_2 and temperature, two main aspects of climate change. It would be useful to expand this study onto mature field grown tea. Studies on the productivity of the crop and the quality of the final product would be beneficial to the industry in general. Metabolomic studies may be utilised with regard to the latter whereby changes in specific compounds under environmental perturbations can be studied and which may reveal impacts on the quality of tea produced.

It is suggested that future work on elevated CO_2 be directed towards long term experimental studies under more natural conditions such as Free Air CO_2 Enrichment (FACE) studies, especially for *C. sinensis*. Furthermore, experiments should focus on interactions with other environmental variables such as soil water availability which are predicted to vary under future climate change.

The variation seen in the *A.thaliana* study may be exploited in order to locate genes responsible for the response to elevated CO_2 and temperature. Furthermore, watering regimes and temperatures that mimic the natural habitats of the ecotypes may provide valuable data on the future fate and management of the natural populations.

Section 4

Impact of research results

i. Relevance of results achieved to scientific advancement

The study identified tea cultivars that showed a high degree of responsiveness to environmental changes in terms of all stomatal variables and those that showed a low overall response.

Tea cultivars showed an increase in leaf thickness with increasing altitude and estate cultivars from each altitude consistently showed a lower palisade to total leaf thickness ratio.

The cluster analysis showed that the estate cultivars sampled in the study were more responsive to their growth environment in comparison to the newly developed TRI cultivars.

The study identified that *A. thaliana* ecotypes showed a differential response in its stomatal density to the natural CO_2 gradient present across an altitudinal range and to the artificial increase in CO_2 in the growth chambers. A novel outcome of this study was the observation that

A. thaliana ecotypes with a higher initial SD were more responsive to elevated temperature. The study confirmed that stomatal size was not as plastic as stomatal numbers in the response of *A. thaliana* ecotypes to both elevated CO_2 and temperature.

The growth chamber study on *C. sinensis* found that SD of *C. sinensis* did not vary due to elevated CO_2 treatment. The GCL decreased showing that stomatal dimensions were responsive to increasing [CO_2]. Conversely, in terms of the response of *C. sinensis* to elevated temperature it was seen that stomatal numbers were more responsive than stomatal dimensions.

ii. Relevance of results achieved to national/socio-economic development

The tea industry has a high socio-economic impact on the country, as it is a major source of foreign income and provides direct and indirect employment to a large number of individuals. Future climate change may affect tea plantations and therefore, timely research is required to understand the response of tea plants to possible climatic changes in the environment. The data from altitude based cultivar studies would be useful for Tea Research Institute in their work to develop cultivars suited to varied environments. Furthermore, the cultivar related data obtained in this project might be utilised in tea breeding programmes or in recommending the most suitable cultivars under future conditions, thereby leading to higher yields.

The study suggests that a 10°C temperature increase would be beneficial for *C. sinensis* and is in agreement with an earlier study based on a crop model for Sri Lankan tea (Wijeratne *et al.*, 2007a). It should be noted that this would be valid only for tea that is at present growing under a non-stressful temperature of around 21°C. However, the study by Wijeratne *et al.* (2007a) showed that tea presently growing at a higher temperature (~28°C) which was already in the upper threshold may be adversely affected by further temperature increases.

The present study showed that the temperature increase led to a decrease in WUE, which may be a concern for a rain fed crop such as tea under predicted water limited scenarios (Rosenzweig *et al.*, 2001; IPCC, 2007).

A majority of the stomatal and anatomical traits reported in this work have not been studied previously and serves to fill a lacuna in information on popular tea cultivars of Sri Lanka.

iii. Dissemination/application of research output

The information on the different tea cultivars would be useful in tea breeding programmes in institutes such as the Tea Research Institute of Sri Lanka, as it identified the responsiveness of cultivars based on their stomatal characters. For instance cultivars such as TRI 3014, TRI 4053 and KEN 16/3 showed the highest degree of responsiveness to environmental cultivars such as TRI 2043 showed an overall limited response to varied environments. Thus, the Sri Lankan tea cultivars may show varied responses in their stomatal characters to future environmental change. Understanding of responses of the Sri Lankan tea germplasm to future climate change would be a significant advantage in breeding new cultivars at the collaborating Institutions.

Section 5

Miscellaneous

i. List of major equipment acquired during the project period and their functionality

Porometer/AP4-UK /Delta-T Devices Limited (130 Low Road, Burwell, Cambridge CB25 0EJ, UK). Used to measure stomatal conductance

ii. List of publications/communications arising from the project and/or presentations made at seminars, workshops etc.

Full-length Paper in Peer-Reviewed Conference/Symposium Proceedings

Caldera H.I.U., De Costa, W.A.J.M., Wijeratne, M.A. and Ranwala, S.M.W. (2011). Response of stomatal characters of Sri Lankan tea cultivars to environmental change. Proceedings of the International Conference on the Impact of Climate Change on Agriculture, 20 December, 2011. (Eds.) M. Wijeratne, N.S.B.M. Atapattu, W.W.D.A. Gunawardena, P.W.A. Perera and N.Y. Hirimuthugoda. pp. 114-123. Faculty of Agriculture, University of Ruhuna, Sri Lanka.

Abstract in Peer-Reviewed Conference/Symposium Proceedings

Caldera, H.I.U., De Costa, W.A.J.M., Woodward, F.I., Lake, J.A., Ranwala, S.M.W.(2012). Stomatal response of *Camellia sinensis* (L.) O. Kuntze to elevated carbon dioxide. Proceedings of the 29th New Phytologist Symposium – Stomata 2012, 2-4 July, 2012, Manchester, United Kingdom. pp. 38.

Caldera, H.I.U. (November 2012). Plant responses to environmental change with special emphasis on anatomical and physiological adaptations of stomata across an altitudinal gradient. PhD dissertation, University of Colombo

Section 6

Summary statement of expenditure - attached

Section 7

i. Grantees signatures

1. Dr. S. M. W. Ranwala 17.10.2014.
2. Prof. W. A. J. M. De Costa 17-10-2014
3. H. I. U. Caldera 17.10.2014

ii. Comments of the Head of the Department/signature

29.10.2014.

HEAD/PLANT SCIENCES
University of Colombo

iii. Head of the Institutions' signature

Dr. W K Hirimburegama
Vice Chancellor
University Of Colombo

කොළඹ විශ්වවිද්‍යාලය, ශ්‍රී ලංකාව
கொழும்புப் பல்கலைக்கழகம், இலங்கை
UNIVERSITY OF COLOMBO, SRI LANKA

20.08.2014

Research Management Unit,
No: 94, Cumarathunga Munidasa Mawata,
University of Colombo,
Colombo 03.

Dr.S.M.W.Ranwala,
Department of Plant Sceince,,
Faculty of Science,
University of Colombo,

Dear Madam,

Statement of Receipt & Payments – RG/2010/BS/01

Please find enclose herewith a statement of Receipts & Payments of the RG/2010/BS/01 for the period ended 30.06.2014.

....

Senior Assistant Bursar
Research Management Unit
University of Colombo

තැපැල් පෙට්ටි අංකය 1490. කුමාරතුංග මුනිදාස මාවත, කොළඹ 3. ශ්‍රී ලංකාව P.O. Box No. 1490, Cumaratunga Munidasa Mawatha, Colombo 3. Sri Lanka
தபாற்பெட்டி இல.1490, குமாரதுங்க முனிதாச மாவத்த, கொழும்பு 3. இலங்கை.

දුරකථන අංක :
தொலைபேசி இல.
Telephone Nos :

Vice-Chancellor	: 2583810	Fax :	2586059	E-mail :	vc@cmb.ac.lk
Registrar	: 2583818	Fax :	5355957	E-mail :	registrar@admin.cmb.ac.lk
Bursar	: 2586652	Fax :	2586652	E-mail :	bursar@admin.cmb.ac.lk
General	: 2581835, 2584695, 2585509				

GRANT NO: RG/2010/BS/01
Statement of Income & Expenditure
as at 30.06.2014

Budget Item	Budgeted Expenditure	Actual Expenditure	Balance
Personal (TA)	60,000.00	-	**60,000.00**
Personal (Other)	93,698.00	93,687.75	**10.25**
Equipment	1,371,263.00	1,318,200.80	**53,062.20**
Consumables	129,500.00	62,112.00	**67,388.00**
Travel & Subsistence	133,125.00	132,825.00	**300.00**
Miscellaneous	40,000.00	35,433.75	**4,566.25**
Total Amount	**1,827,586.00**	**1,642,259.30**	**185,326.70**

Funds received from National Science Foundation

CHQ 117993	22/11/2010	671,263.00	
CHQ 068670	21/10/2010	752,000.00	
CHQ 138255	20/01/2011	118,680.00	
CHQ 418490	20/10/2011	129,610.00	
CHQ 711462	08/04/2013	69,609.00	
CHQ 795705	31/07/2013	86,424.00	
			1,827,586.00
Total Expenditure			1,642,259.30
Balance as at 30.06.2014			**185,326.70**

....

Bursar

....

Senior Assistant Bursar,
Research Management Unit,
University of Colombo.

....

Checked by

Treshika.............................

Prepared by

International Conference on the Impact of
Climate Change on Agriculture, 2011

Proceedings

December 20, 2011
Faculty of Agriculture
University of Ruhuna
Sri Lanka

Response of stomatal characters of Sri Lankan tea cultivars to environmental change

H.I.U. Caldera[a], W.A.J.M. De Costa[b], M.A. Wijeratne[c] and S.M.W. Ranwala[a]

[a]Department of Plant Sciences, Faculty of Science, University of Colombo, Colombo 00300

[b]Department of Crop Science, Faculty of Agriculture, University of Peradeniya, Peradeniya 20400

[c]Tea Research Institute, Low-country Station, Ratnapura.

Abstract

Stomata play a crucial role in linking the atmosphere and terrestrial biosphere through their role in facilitating gas exchange during photosynthesis and transpiration. Stomata are an important aspect of plants' adaptation to climate change. Tea is grown in Sri Lanka at low (<300m) mid (300- 900m) and high (>900m) elevations, providing an altitudinal and natural environmental gradient, within which the response of tea to environmental change can be studied. The stomatal variation present in 20 tea cultivars growing across these altitudes was studied at Ratnapura (29 m amsl), Hantane (762 m amsl) and Talawakelle (1382 m amsl). Stomatal density (SD), stomatal index (SI), guard cell length (GCL) and potential conductance index (PCI) were determined using leaf impressions and light microscopy. Analysis of variance revealed significant ($p<0.001$) altitude x cultivar interactions for all stomatal characters studied, indicating that different cultivars responded differently to variation in altitude. Frequency distributions of the % change of stomatal characters in response to environmental change showed that a majority of cultivars decreased their SD and SI with increasing altitude. This allowed greater CO_2-supplying capacity at lower elevations, where photosynthetic potential is higher because of higher solar radiation and temperatures. Decreasing SD and SI with increasing altitude may be an adaptive response to the lower photosynthetic potential at higher altitudes due to lower temperatures. In contrast to other stomatal characters, GCL of the majority of cultivars increased with increasing altitude and had significant negative correlations with SD and SI. Increased GCL at higher altitudes allowed the plants some degree of flexibility to increase their CO_2-supplying capacity when the environmental conditions are favourable for higher photosynthesis. A minority of cultivars responded to increasing altitude by increasing their SD, SI and PCI and decreasing their GCL, thus showing the complexity of this response probably due to cultivar variation in the responsiveness to environmental change and the interaction of other environmental factors which would change with altitude change.

Keywords: tea, stomatal characters, altitudinal range, cultivar, environmental change

*Corresponding author: irojacaldera@gmail.com

Introduction

Stomata are small pores present on the aerial parts of land flora, predominantly on the abaxial surface of leaves. They play a crucial role in linking the atmosphere and terrestrial biosphere through the inward diffusion of CO_2 during photosynthesis and outward diffusion of water vapour during transpiration. The sessile nature of plants requires them to adapt to variations in their environment and stomata are vital in this process. The manner in which stomata respond to the environment and thereby control photosynthesis and transpiration is an important determinant of plant growth and its water status. Through these two processes, stomata also exert major controls on the global carbon and hydrological cycles and thus areimportant in understanding future climate change. Tea is grown in Sri Lanka at low (<300m) mid (300- 900m) and high (>900m) elevations. Therefore, it provides a natural altitudinal gradient, which also constitutes an environmental gradient, within which the response to environmental change can be studied.In particular, an altitudinal gradient represents a gradient in atmospheric temperature and CO_2 concentration (Gale, 1972), which are two of the key aspects of global climate change. Stomataldevelopment and aperture are controlled by a complex network of intrinsic controls such as plant hormones and ionic fluxes as well as extrinsic environmental signals such as light intensity and atmospheric CO_2concentration (Hetherington and Woodward, 2003). Limited studies have been conducted on stomatal characters of Sri Lankan tea cultivars. In particular, their response to environment change as represented by a gradient in altitude has not been studied previously. Therefore, the objectives of this study were to investigate the cultivar-based variation in stomatal characters of a range of Sri Lankan tea cultivars growing across an altitudinal gradient and, to determine their responsiveness to environmental change.

Materials and Methods

Cultivars

20 tea cultivars were selected with preference being given to those that grew across a wide altitudinal range (Table I). When choosing cultivars, representation was given to the TRI 2000, 3000 and 4000 series as well as estate selections.

Study sites

Sampling was carried out in the tea plantations maintained at the three regional stations of the Tea Research Institute of Sri Lanka at Ratnapura(6° 41' N, 80° 40' E, 29m amsl), Hantane, Kandy (7° 26 N', 80° 38' E, 762m amsl) and Talawakelle (6° 55' N, 80° 40' E, 1382m amsl). The rainfall and temperature data for the study sites are given in Table 2.

Sample collection, preparation and measurements

Three mature leaves (mother leaves) each was collected from five different plants from each cultivar at each location. Negative impressions of the abaxialsurface of the mid leaf lamina were taken with high precision Polyvinylsiloxane (Coltene-Whaledent Ltd, UK). Positive impressions were then made using clear cellulose varnishand mounted onto microscope slides. The number of stomata and epidermal cells were counted under light microscopy (LeitzLaborlux S, Leitz, Germany) using a calibrated eye piece graticule. The length measurements were carried out using a Quantimet 500 image analysis system (Leica, Milton Keynes, Middlesex, UK) coupled to a light microscope. Five fields of view per leaf sample were randomly selected for microscopy. The following characters were determined: Stomatal density (SD) as the number of stomata per unit area (mm^{-2}); Stomatal index (SI) calculated as {(stomatal density)/[stomatal density + epidermal cell density] x 100}; Guard cell length (GCL); Potential Conductance Index (PCI) as {[guard cell length]^{2}x stomatal density x 10^{-4}}.

Data analysis

The percentage change of a given stomatal character to increasing altitude was calculated as: % change = $[(SCV_H - SCV_L)/SCV_L]$ x 100, where, SCV_H and SCV_L are the respective cultivar means of the stomatal character at higher and lower altitudes. Frequency distributions of the magnitude of % change of each stomatal character were generated. As all cultivars were unavailable at the three elevations, the analysis of variance with the GLM (General Linear Model) procedure of SAS - version 9.0 (SAS Institute., Cary, N.C) was used to detect significance of variation of stomatal characters with altitude and cultivar. Duncan's Multiple Range Test (at $p < 0.05$ level) was used for multiple comparisons amongst means.

Results and Discussion

Analysis of variance revealed significant ($p<0.001$) altitude x cultivar interactions for all stomatal characters studied, indicating that different cultivars responded differently to the environmental change represented by the altitudinal gradient (Table 3).

Table I. Cultivars used for the study and the sampling locations

	Cultivar	Ratnapura (29m amsl)	Hantane (762m amsl)	Talawakelle (1382m amsl)
1.	TRI 2025	X	X	X
2.	TRI 2016	X		
3.	TRI 2026	X		
4.	TRI 2027	X	X	
5.	TRI 2043		X	X
6.	TRI 3025	X		X
7.	TRI 3055	X		X
8.	TRI 3014	X		X
9.	TRI 3019		X	X
10.	TRI 3016			X
11.	TRI 4042	X		X
12.	TRI 4049	X		X
13.	TRI 4053	X		X
14.	TRI 4052			X
15.	KEN 16/3	X	X	
16.	H1/58	X		
17.	CY 9		X	X
18.	PK 2		X	X
19.	DN		X	X
20.	DT 1			X

Table 2. Key climate data of the study sites

Location	Elevation range	Annual rainfall (mm)	Maximum temperature (^{0}C)	Minimum temperature (^{0}C)
Ratnapura	Low	3617	32.0	22.9
Hantane	Mid	1863	29.0	20.2
Talawakelle	High	2250	22.8	14.2

Table 3. Variance ratios (*F* – values) and levels of significance (* $p < 0.05$; ** $p < 0.01$; ***$p < 0.001$; ns – not significant at $P > 0.05$) following analysis of variance (GLM) of stomatal characters of selected *Camellia sinensis* cultivars sampled from three altitudes.

		F-value			
Sources of variation	d.f.	SD	SI	GCL	PCI
Cultivar	19	77.83***	56.02***	52.17***	36.95***
Location	2	132.77***	63.68***	8.76***	62.3***
Cultivar x location	13	17.65***	7.52***	7.28***	7.73***

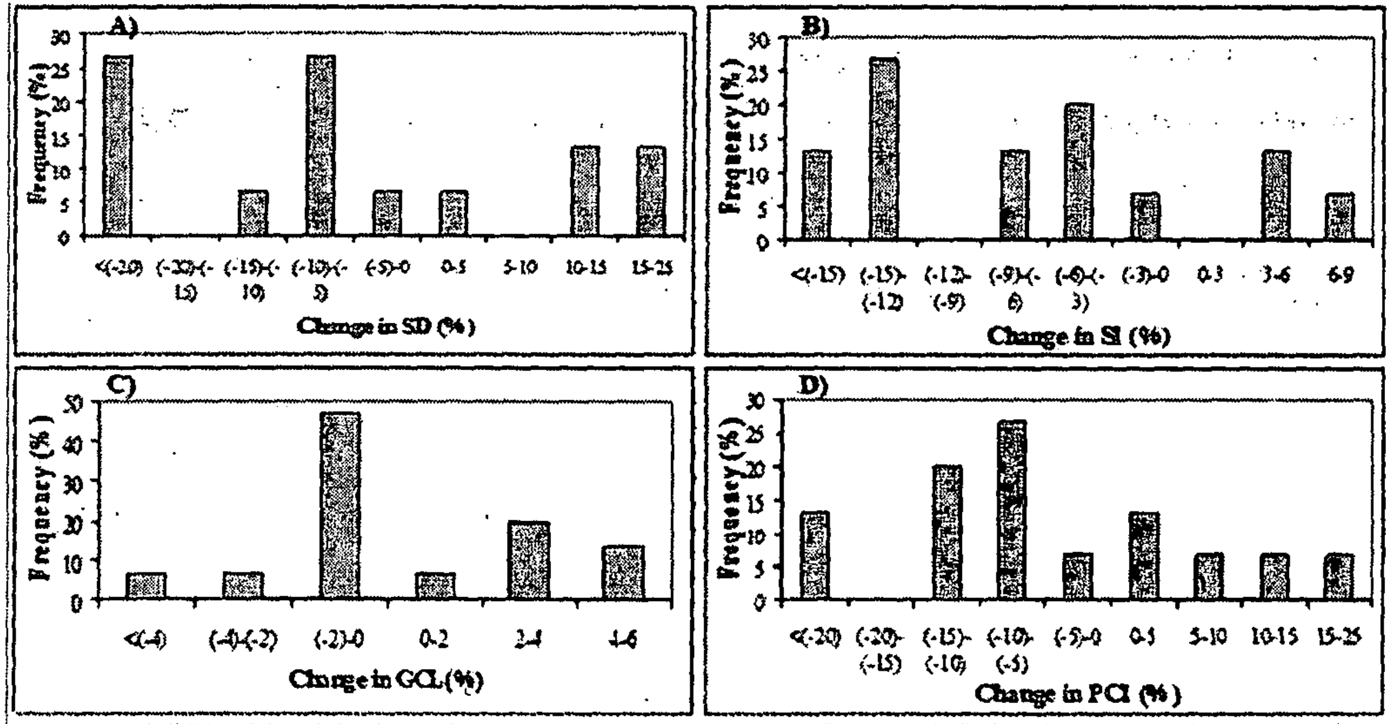


Figure 1. Frequency distribution of the percentage change in stomatal characters with increasing altitude.Frequency is the % of cultivars showing a given % change in a stomatal character. A)stomatal density; B) stomatal index; C) guard cell length; D) potential conductance index.

Fig. 1 shows the frequency distributions in the percentage change in stomatal characters with increasing altitude. A majority of cultivars (i.e. a greater frequency) showed reductions in SD, SI and PCI in response to increasing altitude. On the other hand, a majority of cultivars showed a response within ± 2% change in GCL. An increase in altitude represents an environmental change in terms of decreasing atmospheric CO_2 (C_a) and air temperature. Our observations of both increases and decreases of SD in response to increasing altitude agree with similar observations by Woodward and Kelly, (1995) and Hetherington and Woodward, (2003) for a wide range of natural plant species in terms of their response to increasing C_a. On the other hand, clear decreases in SD with increasing C_a have been observed for a smaller number of plant species (Woodward 1987) and for different ecotypes of the model plant *Arabidopsis thaliana* (Woodward et al., 2002). Our observation of a majority of tea cultivars tested showing reductions in SD with increasing altitude, i.e. with decreasing C_a (Fig. 1A), is in direct contrast to the trend observed by Woodward (1987) and Woodward et al. (2002). There can be several reasons for these contrasting observations. Woodward (1993) showed that decreasing SD with increasing $C_a$conferred a competitive and selective advantage to plants by increasing water use efficiency (WUE). However, increased WUE may confer an advantage only in environments where water is a limiting factor. All three locations used in the present study are within the wet zone and receive an annual rainfall in excess of 1800 mm (Table 1), which is well-distributed within the year with no prolonged rainfree periods. Therefore, there would be no ecological advantage for the tea crops to have a higher WUE by having lower SD at the low altitude (i.e. high C_a) and progressively increase SD with increasing altitude (i.e. decreasing C_a). Instead, it may be more advantageous to develop higher SD at the lower altitude to increase the CO_2 absorption capacity and maximize photosynthetic rates by utilizing the higher solar radiation and temperature levels that prevail at the lower altitudes. In contrast, the photosynthetic potential of tea crops would be lower at the higher altitudes because of lower temperatures (Table 1). Accordingly, there would not be any ecological advantage for tea crops growing underthe lower C_a levels at higher altitudes to increase their SD and increase the CO_2-suplying capacity. Therefore, results of the present study indicate that in tea, the influence of decreasing temperature in restricting photosynthetic capacity exerts a greater control in determining the variation of SD and its associated stomatal characters than the influence of decreasing C_a in attempting to increase the CO_2-supplying capacity. However, as demonstrated by the minority of cultivars, which have shown increases in SD with increasing altitude, the response of stomatal characters of tea to environmental change may have complex interactions with genetic factors as well as environmental factors other than temperature and C_a. This is in agreement with the conclusions of Woodward et al. (2002) and Hetherington and Woodward (2003).

The significant negative correlation between SD and GCL (Table 4) show that a reduction in the CO_2-supplying capacity due to a reduction in SD can be partially compensated by a larger size of the stomatal pore (as indicated by higher GCL). A similar negative relationship between SD and GCL has been observed by Hetherington and Woodward (2003) as well for a wide range of plant species. This negative relationship introduces some degree of plasticity to the response of stomatal characters to environmental change. Even when SD is lower because of lower photosynthetic potential in a given environment (e.g. higher altitudes), higher GCL allows the plants some degree of adaptation to increase their CO_2 supplying capacity during time periods when the photosynthetic potential of the environment is higher (e.g. during mid-day when temperatures are higher and are within a more favourable range for higher photosynthesis).

Table 4. Pearson Correlation coefficients between stomatalcharacters of selected *Camellia sinensis*cultivars sampled from three altitudes (N=2625)

	SD	SI	GCL
SI	0.90***		
GCL	-0.38***	-0.26***	
PCI	0.77***	0.75***	0.28***

The specific cultivars that showed significant variation in stomatal characters due to location are shown in Fig 2.The response of SD of cultivar TRI 3019, TRI 3020, DN and PK2 to altitude changes was similar to that originally proposed by woodward (1987). The rest of the cultivars, which included cultivars from 2000, 3000 and 4000 series as well as estate selections showed a trend which was opposite to that reported by Woodward (1987). However, it should be noted that Woodward (1987)'s conclusions were based on SD measurements on herbarium specimens of eight temperate arboreal species, which were up to 200 years old. Hence, reductions in SD in response to increasesin C_a that occurred during the last 200 years as observed by Woodward (1987) constitute a long-term response of stomatal characters to environmental change. In contrast, all measurements of the present study were made on plant samples which had been exposed to variations in environment (e.g. increases in temperature and C_a along with other associated changes) over a shorter time scale (i.e. < 20 years). This could be another reason for the majority of cultivars of the present study not behaving according to Woodward (1987)'s originally-observed pattern of response. Furthermore, the sensitivity and the capacity to respond to environmental change may differ between cultivars of tea. This could be another reason for the significant diversity in the response of stomatal characters to environmental change as observed in the present study. Further experiments are underway to elucidate the physiological and ecological basis of this vital response.

A higher number of cultivars showed a significant response in their SD than their SI. Changes in SI are independent of the stage of expansion or the size of the epidermal cells. Hence,cultivars in Fig. 2B show a significant response in their stomatal development itself (Woodward, 1987) when grown in different environments. The cultivars TRI 3014, TRI 4053 and KEN 16/3 showed the highest degree of responsiveness to environmental change as they showed significant variation in all stomatalvariables measured (Fig. 2). Therefore, these cultivars have the genotypic potential to alter both their stomatal numbers and dimensions in response to future climate change scenarios. Cultivars such as TRI 2025, TRI 3019, TRI 3055 and CY9 varied their SI but the stomatal dimensions were relatively stable (Fig. 2B and 2C). Conversely cultivars such as TRI 2043 showed an overall limited response to varied environments. Thus, the Sri Lankan tea cultivars may show varied responses in their stomatal characters to future environmental change. Such diversity of responses to future climate change within the Sri Lankan tea germplasm would be a significant advantage in breeding new cultivars which are better adapted to future climates.

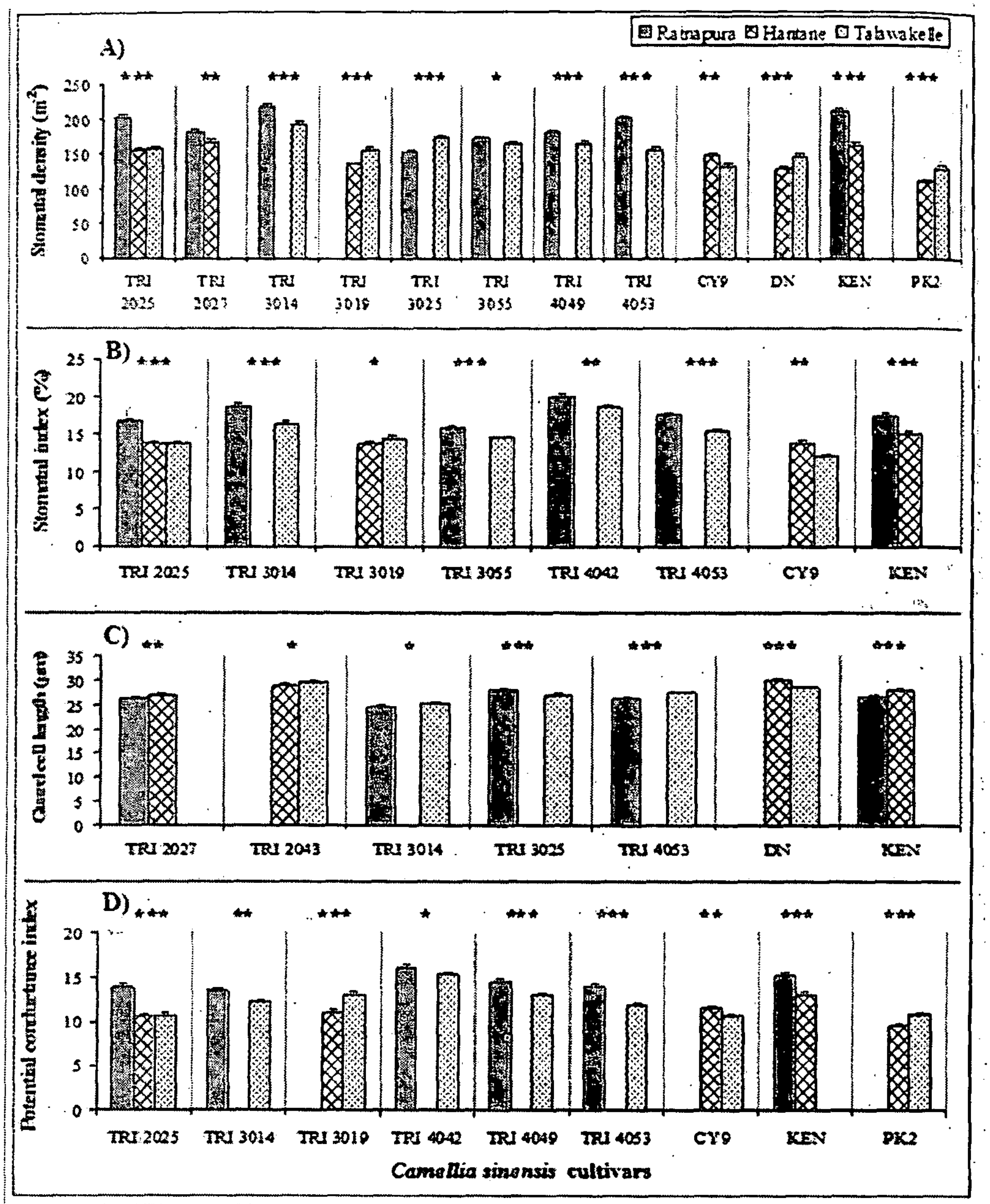


Figure 2. *Camellia sinensis*cultivars that showed significant variation in stomatal characters with environmental change.A) stomatal density; B) stomatal index C) guard cell length; D) potential conductance index. Error bars = SE mean. *n*= 75. (Significant at*p< 0.05; **p< 0.01; ***p< 0.001)

Conclusions

Based on the results of this study, it can be concluded that stomatal characters of tea respond to environmental change as represented by an altitudinal gradient in order to maximize the CO_2-supplying capacity as determined by the prevailing environmental conditions at different altitudes. However, there is a significant diversity in this response within the Sri Lankan tea germplasm probably due to genetic factors as well as due to interactions with other environmental factors.

References

Gale J 1972 Availability of CO2 for photosynthesis at high altitudes: theoretical considerations. Ecology 53:,494-497

Hetherington A M and Woodward F I 2003 The role of stomata in sensing and driving environmental change. Nature. 424: 901-908

Woodward FI, Kelly CK. 1995. The influence of CO2 concentration on stomatal density. New Phytologist.131: 311-327

Woodward F I, Lake J A, Quick W P 2002 Stomatal development and CO2: ecological consequences. New Phytologist. 153: 477-484

Woodward F I 1993 Plant responses to past concentrations of CO2. Vegetatio 104/105: 145-155

Woodward F I 1987 Stomatal numbers are sensitive to increases in CO2, from pre-industrial levels. Nature. 327: 617-618

National Digitization Project

National Science Foundation

Institute : National Science Foundation

1. Place of Scanning : Sanje (Private) Ltd, Hokandara
2. Date Scanned : 2017/04/18
3. Name of Digitizing Company : Sanje (Private) Ltd, No 435/16, Kottawa Rd,
   Hokandara North, Arangala, Hokandara
4. Scanning Officer

Name : H.P.A.V. Caldera

Signature :

Certification of Scanning

I hereby certify that the scanning of this document was carried out under my supervision, according to the norms and standards of digital scanning accurately, also keeping with the originality of the original document to be accepted in a court of law.

Certifying Officer

Designation : Information Officer

Name : Renuka Sugathadasa

Signature : K.P. Sugathadasa

Date :

"This document/publication was digitized under National Digitization Project of the National Science Foundation, Sri Lanka"