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Elevated Co_2 Influences Physiological Responses and Assimilatory Functions on Clones of *Eucalyptus camaldulensis*

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ABSTRACT

Response of selected clones of Eucalyptus *(Eucalyptus camaldulensis* Dehnh.) was measured to elevated CO_2 concentrations over a period of six months. Plants were exposed to double the atmospheric CO_2 concentrations in open top chambers. Responses measured included changes in concentrations of carbohydrate, chlorophyll, total protein in addition to net photosynthetic rate and growth over a period of six months. The study revealed CO_2-induced increases and differences in the response of different clones of *E. camaldulensis.* The results showed that elevated CO_2 had a significant influence on all the biochemical parameters. A significant increase was observed in total biomass of the plants, though only shoot development was enhanced significantly as a result of elevated CO_2. Root biomass was not affected by enhancement of CO_2. At the same time, internal CO_2 levels and stomatal conductance significantly varied, suggesting that intercellular CO_2 concentration might be an important determinant of photosynthetic acclimation in this species. Among the four productive clones released for commercial use by IFGTB, clone EC 1 was found most resilient to elevated CO_2 and temperature levels suggesting it's suitability for planting in anticipation of future oncreased in atmospheric CO_2 levels.

Key words:

Eucalyptus, biochemical parameters, biomass production, net photosynthetic productivity, OTC

INTRODUCTION

Increases in atmospheric CO_2 concentrations and the associated rise in temperature and precipitation patterns will have profound effects on terrestrial plant growth and productivity in the near future. According to the Intergovernmental Panel on Climate Change (IPCC 2007), levels of carbon in the atmosphere has risen from 285 μmol l^{-1} (600 gigatonnes (Gt)) in the pre-industrial times to the current level of 384 μmol l^{-1} (800 Gt) and the predicted rise in the atmospheric CO_2 would approach 1000 Gt by the year 2050. The increase in CO_2 concentrations as well as other greenhouse gases, due to anthropogenic intensification, will result in increased global average temperatures that may then cause drastic shifts in precipitation patterns (Reddy and Gnanam 2000; Chaplot 2007). This warrants an urgent need to understand the synergistic and holistic mechanisms associated with plant growth and productivity under e CO_2 (eCO_2) concentrations.

Climate change affects plant growth and development primarily due to changes in photosynthetic carbon assimilation patterns. Physiological processes are the critical intermediaries through which heredity and environment interact to regulate plant growth. Tree species show high genetic variation in size, crown form, longevity, growth rate, cold hardiness, and tolerance to environmental stresses. Trees are subjected to multiple abiotic and biotic stresses that affect growth by influencing physiological processes. Environmental stresses set in motion a series of physiological disturbances that may adversely affect growth. In-depth knowledge of the physiology of woody perennials provides deeper insights into the complexity and control of plant growth which supports useful application of this information in efficient measurements of these varied responses (Warrier 2010).

The acclamatory responses of plants to the rapidly changing environment and understanding the potential impacts of multiple interacting factors (water availability, temperature, soil nutrition and ozone) have become a subject of debate over the past two decades. Conflicting reports on plant responses to CO_2, and several such differential photosynthetic responses, could be attributed to differences in experimental methods, plant species used for the experiments, age of the plant as well as duration of the treatment (Sage 2002; Davey et al. 2006; Raj et al. 2014; Sharma et al. 2016).

The effects of CO_2 on C3 photosynthetic rates have been the subject of many CO_2 enrichment studies and these studies show that photosynthetic rate is increased following initial exposure to CO_2 (hours to days) (Farquhar and Sharkey 1982; Pearcy et al. 1987). Increases in photosynthetic rate are brought about by increased availability of CO_2 at the chloroplasts and reductions in photorespiration resulting from an increased ratio of CO_2 to O_2 in the sub-stomal space (Pearcy et al. 1987). Short-term exposure of C3 plants to CO_2 often stimulates photosynthesis (Gifford 1992), producing significant gains in biomass as a result of the improved competitiveness of CO_2 over O_2 as a substrate for the main C3 photosynthetic enzyme, ribulose-l,5-bisphosphate carboxylase- oxygenase (Rubisco) (Bowes 1993). Plants grown in CO_2 can show a degree of photosynthetic acclimation (Besford et al. 1990) such as an increase or more commonly a decrease in photosynthetic performance as compared to plants grown in low (ambient) concentrations of CO_2, due to intrinsic changes in the photosynthetic machinery (Gunderson and Wullschleger 1994).

However, many studies report that these high initial photosynthetic rates are not maintained over long time periods. Substantial reductions in photosynthesis may occur within days to weeks after initial exposure to e CO_2 (Long et al. 1993; Sims et al. 1998). Photosynthetic down-regulation is characterized at the biochemical and leaf levels by reduced chlorophyll content, reduced Rubisco content and activity, limitations in (ribulose-1,5-biphosphate) RuBP and Pi regeneration, higher leaf mass/leaf area ratios and decreased leaf nitrogen concentration on a leaf mass basis (Sage 1994; Tissue et al. 1995).

In India, studies using Open Top Chambers (OTCs) to understand the synergistic and holistic mechanisms associated with plant growth and productivity in relation to global e CO_2 concentrations began in 1995. However, ,mainly the food crops such as rice and brassica (*Brassica juncea*) (Upreti et al. 2000), castor bean (*Ricinus communis* L.) and blackgram (*Vigna mungo*), Greengram (*Vigna radiata*) (Srivastava et al. 2001), sorghum (*Sorghum vulgare*) and sunflower (*Helianthus annuus*) have been focused on for their response to e CO_2 (Vanaja et al. 2006). Tree species have received very scant attention in this regard in India. Raj et al. (2014) recommend that studies related to AOTCs should not generalise the response of tropical tree species to elevated CO_2. Instead, they should be assessed individually for the physiological functions to elevated CO_2 to understand variations within individuals within a species.

The plantation forests of India account for 17% of global plantations, and are the second

largest in the world after those in China. India is also the largest planter of Eucalyptus in the world. Thus, the productivity of this species must be investigated under varied climatic conditions. Our paper aims to describe the effects of CO_2 on aboveground growth, physiological and biochemical parameters of selected clones of *Eucalyptus camaldulensis* Dehnh. using OTC conditions, and to relate the observed differences in photosynthetic CO_2 uptake to underlying biochemical characteristics and assimilatory functions in this economically important tree species.

MATERIALS AND METHODS

First generation provenance trials using seeds from CSIRO, Australia were established in ten different locations and about 100 clones of *E. camaldulensis* were selected, based on individual tree superiority for height, diameter at breast height and straightness of stem through index selection method. The clonal trials were established in three different locations: Coimbatore (11° 00' N, 76° 58' E, 400 m altitude, 900 mm annual rainfall), Sathyavedu (13° 25' N, 79° 57' E, 215 m altitude, 1150 mm annual rainfall) and Kulathupuzha (8° 50' N, 77° 15' E, 230 m altitude, 2800 mm annual rainfall). Thirty-three clones across all the three trials were compared with 10 commercial clones and seed origin plants of *E. camaldulensis* to prove clonal superiority. The top four clones that showed consistent performance in all the three trials over the control were designated IFGTB EC1, IFGTB EC2, IFGTB EC3 and IFGTB EC4selected, released for commercial use and selected for the present study. Ten replicates of each clone were used in each of the treatments (n=10 for all measured parameters).

The selected four clones (two month old) were grown at Coimbatore inside OTCs 3 m in diameter and 10 m in height lined with transparent PVC sheets (0.125 mm thickness). Pure CO_2 gas was used for the enrichment. Rubber pipes with small holes throughout were circulated inside the OTC, which acted as the e CO_2 environment and the same was connected to the gas cylinders containing pure CO_2 gas. The flow of the CO_2 was adjusted with a flow meter to get the exact concentration of CO2 (600 ±50 ppm). Similarly OTCs were used as control where the clones were grown under ambient CO_2 (360 ppm). The clones were also grown in open field with ambient CO_2 (360 ppm). The experiments were laid in a Complete Randomized Design. The three treatment combinations were: (i) "Ambient" (ii) Chamber control "OTC" and (iii) "eCO_2" (elevated [CO_2]).The period of CO_2 enrichment was 180 days after the initial acclimatization period. Supervisory Control and Data Acquisition (SCADA) was used to continuously control, record and display the actual and desired CO_2 level, relative humidity and temperature in each OTC by feedback control loop passing through Programmable Logical Controllers (PLC) throughout the study period (Buvaneswaran et al. 2010).

Measurements of photosynthesis and related parameters

The net photosynthetic rate (Pn), stomatal conductance (gs), intercellular CO_2 concentration (Ci) and transpiration rate (E) were measured with a LI-6200 portable photosynthesis system (LICOR, Inc, Lincoln, NE, USA). The measurements were taken between 9.30 am and 11.30 am under cloud free conditions. Three observations each from ten ramets per clone were recorded for all the physiological parameters. Water Use Efficiency (WUE) was also estimated from the clones. Intrinsic water use efficiency was estimated as the ratio of net photosynthetic rate to stomatal conductance (Pn/gs), whereas instantaneous water use efficiency was estimated as the ratio of net photosynthetic rate to transpiration (Pn/E). Intrinsic carboxylation efficiency was derived as the ratio of net photosynthetic rate to intercellular CO_2 concentration (Pn/Ci). Intrinsic mesophyll efficiency was estimated as the ratio of intercellular CO_2 concentration to stomatal conductance (Ci/gs) (Sheshayee et. al. 1996).The leaves and stem portion were separated after the recording of the plant height and total number of leaves. All the plant parts were dried at 80°C for determining the dry mass. The fresh and dry masses of the plant samples were recorded.

Biochemical analysis

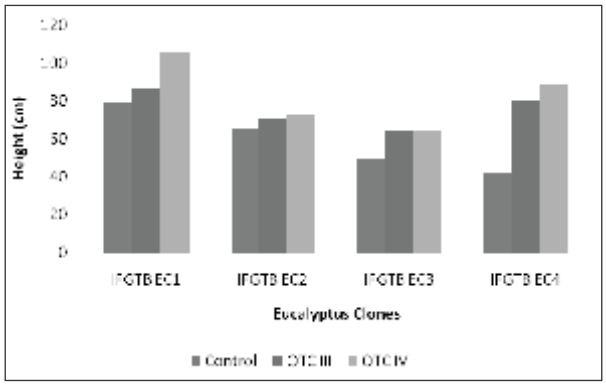
To determine chlorophyll content, apical leaves were collected at random from four ramets per clone. After cleaning, the leaves were cut into small pieces, pigments extracted in 80 % acetone (v/v), and measured colorimetrically with a UV-VIS spectrophotometer (Labtronics, India) at 645, 654 and 663 nm. Chlorophyll (a,b and total) content on a fresh mass basis was calculated using the method of Yoshida et al. (1976). For soluble protein estimation fresh leaves were ground in a pre-chilled pestle and mortar with 1:2 (m/v) 50 mM potassium phosphate buffer, pH 7.0 and the homogenate was centrifuged at 4°C for 20 min. at 15000 g. The supernatant was used for estimating soluble protein following the procedure of Lowry et al. (1951). Total carbohydrates were extracted into solution by acidifying leaf samples with 1.5 N HCl, and the neutralized supernatant was estimated colorimetrically using anthrone method (Hedge and Hofreiter 1962). Free reducing sugars were estimated by method of Miller (1972).

Statistical analysis

Data were subjected to two-way analysis of variance (ANOVA) to assess the effects and interactions of treatment and clones, using SPSS data analysis software (Version 16, IBM Corporation). Values presented are means ± SE. All statistically significant differences were tested at the $p < 0.05$ level. Prior to statistical analyses, variables were checked for normality (using the standard Shapiro-Wilk Test) and transformed wherever necessary.

RESULTS AND DISCUSSION

Exposure to CO_2 in open-top chambers increased the growth rate of Eucalyptus clones. Plant height and shoot (stem and leaf) biomass increased in CO_2 grown plants significantly. Among the clones, EC 1 attained a maximum height of 105.8 cm, followed by EC 4 (88.6 cm), EC 2 (73 cm) and EC 3 (63.9 cm) under eCO_2 at the end of six months and EC1 was 35% higher than the control (Fig. 1).



Control = Ambient condition, OTC III =Open top chamber with ambient CO_2, OTC IV = Open top chamber with e CO_2,

Figure. 1. Effect of elevated CO2 on the height of Eucalyptus clones

A significant increase ($p<0.05$) in the leaf number was observed among the clones under eCO_2 (Table 1). The number of leaves increased from 8.4 to 25.2 over the six month period and the maximum increase was observed in clone EC 3. The shoot biomass increased significantly in all the clones except EC2 when the plants were grown under high concentration of CO_2 (Table 1).

Table 1. Plant height, dry weight and number of leaves in Eucalyptus clones at the end of six months experiment

Clones	Plant height (cm)			Biomass (gm^{-1})			No. of leaves		
	OPEN	OTC	OTC+CO_2	OPEN	OTC	OTC+CO_2	OPEN	OTC	OTC+CO_2
IFGTB EC1	78.8 ± 15.4	105.8 ± 17.6	86.8 ± 12.5	12.62 ± 5.02	16.06 ± 5.05	15.29 ± 4.12	23 ± 3.3	33 ± 10.4	24.2 ± 5.5
IFGTB EC2	49.1 ± 4.9	64.2 ± 9.4	63.9 ± 8.0	8.95 ± 2.40	7.09 ± 2.00	7.44 ± 2.16	17.2 ± 4.7	21.4 ± 3.6	19.6 ± 2.6
IFGTB EC3	65.8 ± 4.9	70.6 ± 10.9	73.0 ± 14.2	6.99 ± 0.78	11.22 ± 2.06	10.78 ± 1.58	29.6 ± 4.8	22.2 ± 6.4	20.6 ± 4.5
IFGTB EC4	41.6 ± 7.4	80.2 ± 24.4	88.6 ± 12.6	5.59 ± 0.98	15.58 ± 19.92	14.29 ± 3.82	19.2 ± 5.7	35.6 ± 2.7	19.4 ± 6.1
P VALUES p<0.05	C=46.85 T =20.69 C x T =5.33			C=3.77 T =3.64 C x T =3.28			C= 3.46 T = 6.52 C x T=1.69		

OPEN = Ambient condition, OTC =Open top chamber with ambient CO2, OTC+CO2 = Open top chamber with e CO2, T = Treatment, C = Clone, Values significant at $p<0.05$ level

Photosynthetic rates

The net leaf photosynthetic rate (Pn) of plants grown at eCO_2 was significantly decreased ($P < 0.05$) by about 25 to 60 per cent for *Eucalyptus clones* compared with that of plants grown at ambient CO_2 (Table 2). The photosynthetic rates differed significantly between the clones ($P < 0.05$), with significant interaction between CO_2 concentration and clones over a period of six months. All the four clones showed a reduction in the levels of Pn when subjected to e CO_2, while Ci showed variations. Clones EC 2 and EC 3 were able to maintain a higher concentration of CO_2 within tissues, while EC 1 and EC 4 had concentrations lower than those of the control.

Chlorophyll Content

Elevated CO_2 increased chlorophyll a, b and total chlorophyll in plants over the control (Table 3), which suggests an increase in efficiency of radiant energy capture through a shift in carbon allocation. The accumulation of soluble protein in the eucalyptus leaves decreased under e CO_2 in all the clones except EC1 where significant increase was recorded under e CO_2 levels. A reverse trend was observed in total carbohydrates. Except EC1, all the clones recorded a decrease in the levels of total carbohydrates. (Table 3).

Table 2. Photosynthetic parameters in Eucalyptus clones at the end of six months as influenced by CO_2

Clones	Pn			Gs			Ci			E		
	OPEN	OTC	OTC+CO$_2$	OPEN	OTC	OTC+CO$_2$	OPEN	OTC	OTC+CO$_2$	OPEN	OTC	OTC+CO$_2$
IFGTB EC1	9.82	2.47	3.23	0.146	0.064	0.020	322.36	342.76	134.65	4.31	2.75	0.92
IFGTB EC2	4.07	2.37	2.37	0.056	0.030	0.056	215.88	178.73	260.47	2.18	1.98	2.77
IFGTB EC3	2.44	0.60	1.81	0.022	0.044	0.044	180.99	326.73	254.32	1.16	2.12	2.47
IFGTB EC4	3.36	0.43	1.72	0.054	0.050	0.022	234.71	358.47	191.43	2.53	1.10	1.33
P VALUES		C	23.04		C	2.77		C	4.65		C	4.43
p<0.05		T	3.78		T	NS		T	11.37		T	NS
		CxT	7.75		CxT	6.02		CxT	8.79		CxT	4.18

OPEN = Ambient condition, OTC =Open top chamber with ambient CO2, OTC+CO2 = Open top chamber with e CO2,
T = Treatment, C = Clone, Values significant at p<0.05

As the air's CO_2 content continues to rise, *Eucalyptus* seedlings will probably display enhanced rates of photosynthesis and biomass production. Thus, young *Eucalyptus* trees will likely sequester ever more carbon within their woody tissues as time progresses (Kirdmanee et al. 1995).

As suggested by Gleadow et al. (1998), with increasing atmospheric CO_2 concentration, Eucalyptus will probably experience a slowdown in photosynthetic regulation without significantly affecting the growth stimulation brought about by e CO_2. This phenomenon would thus lead to larger eucalypts trees with better-developed root systems. In addition, increasing levels of CO_2 would allow batter allocation of the nitrogen allocation for mobilization into leaf defence components so that this species can maintain a stable degree of protection as the CO_2 content of the air rises ever higher.

Though there was an overall increase in the plant biomass, the root biomass did not

Table 3. Biochemical parameters in Eucalyptus clones at the end of six months as influenced by CO_2

Clones	Chlorophyll a			Chlorophyll b			Total Chlorophyll			Chlorophyll a:b ratio		
	OPEN	OTC	OTC+CO$_2$	OPEN	OTC	OTC+CO$_2$	OPEN	OTC	OTC+CO$_2$	OPEN	OTC	OTC+CO$_2$
IFGTB EC1	0.53	0.64	0.64	0.28	0.33	0.34	0.81	0.96	0.98	0.81	0.96	0.98
IFGTB EC2	0.94	0.52	1.30	0.27	0.28	0.68	1.21	0.8	1.98	1.21	0.80	1.98
IFGTB EC3	1.00	0.53	1.64	0.27	0.34	0.74	1.27	0.87	2.38	1.27	0.87	2.38
IFGTB EC4	0.71	0.42	0.62	0.23	0.22	0.29	0.94	0.64	0.91	0.94	0.64	0.91
P VALUES		T	7.30		T	6.40		T	8.2		T	5.67
p<0.05		C	65.01		C	6.45		C	33.16		C	2.78
		TxC	7.90		TxC	NS		TxC	3.64		TxC	NS

OPEN = Ambient condition, OTC =Open top chamber with ambient CO_2, OTC+CO_2 = Open top chamber with e CO_2, T = Treatment, C = Clone, Values significant at p<0.05

significantly change under eCO_2 suggesting that CO_2 elevation enhanced carbohydrate assimilation resulting in increased height and shoot biomass but did not affect the roots. This result supports the observations of Sharma and Sengupta (1990), which showed that the extra carbon fixed by the plants due to CO_2 enrichment translocated towards the growing axis. In our experiment, high CO_2 stimulated increased number of leaves per plant and allocated more biomass to stem and leaves (Table 1). According to Poorter et al. (1979) this pronounced increase in biomass is due to changes in leaf chemical composition, mainly due to the accumulation of total nonstructural saccharides. Leaf area index (leaf area/land area) has been observed to increase with increases in the number of leaves, resulting in higher carbon assimilation on an ecosystem level in Pinus sylvestris seedlings grown at eCO_2 (Jach and Ceulemans 1999). From our finding, we also confirmed that the biomass in all the genotypes increased significantly when the species was subjected to e CO_2 environment (Fig. 2). Ceulemans et al. (1996) reported that poplar clones exhibited different and significant positive responses to eCO_2 resulting in increased investment in branch and leaf biomass. However, this is contrary to a recent report by Reddy et al. (2010) who noted that increased root volume in *Gmelina arborea,* a tropical tree species, under eCO_2 where the saplings were the growing conditions differed. In this case, the plants were planted inside the chamber.

IFGTB EC3 and 4 showed an increase in dry biomass, which is in agreement with earlier results (Uprety et al. 2000; Vanaja et al. 2006). This could be attributed to the marginal increase in the temperature in the chamber. Elevated CO_2 stimulated total dry biomass accumulation in all the Eucalyptus clones except EC2. Steady increase of dry matter is a common physiological response to high CO_2 concentration (Atkinson et al. 1997). C3 plants have been observed to show increase in shoot biomass (Rasse et al. 2005) as well as total plant biomass (Poorter and Perez-Soba 2001) when grown at e CO_2, at least in the short term and at non-limiting nutrient conditions.

Photosynthetic parameters

Figure 2 demonstrates the Pn/Ci curve relation in *Eucalyptus* clones as a result of e CO_2 levels. These parameters (pn, Ci and Pn/Ci) are commonly used when monitoring stress sensitive photosynthetic characteristics. The changes in PN under e CO_2 are often associated with altered ribulose-1,5-biphosphate carboxylase/oxygenase content (Stitt 1986). Fig 2 demonstrates the changes observed in Eucalypus clones with the Pn-Ci curve under open field, ambient CO_2 in OTC and OTC with e CO_2 levels. The control clones have been depicted as empty circles, the clones in OTC

under ambient CO_2 as partially shaded, and the clones in e CO_2 levels as fully shaded. It was observed that there was a corresponding change in the stomatal conductance values also, the relationship between the two being depicted as significant negative correlation (r=-0.81**). Decreased Pn during growth could be interpreted in terms of high CO_2 induced transient inactivation of photosynthesis as a stress response (Lichtenthaler 1996). The Pn decreased under OTC (without e CO_2) also in all the clones (Table 2). The stomatal conductance followed similar pattern as Pn. Stomatal conductance is of utmost importance when photosynthesis is concerned as the stomates play a pivotal role in controlling the balance between assimilation and transpiration (Beadle et al. 1981). According to Harley et al. (1992) stomatal conductance (gs) decreased in e CO_2. The reduction in Pn under e CO_2 may be due to lower stomatal conductance, which also declined under e CO_2 levels in EC 1 and EC 4, while it was higher and 100 per cent more in EC 2.

The role of stomata in determining the water use efficiency is well understood (Leverenz et al. 1999; Li 2000). The genotypes that can maintain higher water use efficiency will have an efficient stomatal regulatory capacity (Maroco et al. 1997). A higher instantaneous WUE value indicates better efficiency of the plant to divert water for photosynthesis than transpiration. Measurement of WUE might be a useful trait for selecting genotypes with improved drought adaptation and biomass productivity under different environmental conditions (Li 2000). In our study (Fig. 3), it was observed that though clones EC 2 and EC 3 had the highest WUE under control conditions, they showed poor WUE under CO_2 levels. The other two clones namely, EC 1 and EC 4 were able to demonstrate better WUE over the control under high CO_2 levels, EC1 showing almost 200 per cent increase. This could be attributed to the genotypic variations in the clones which could be responsible for this adaptive variation within the species. Zhang and Marshall (1994) reported genotypic differences in long-term measures of instantaneous WUE among the native populations of *Larix occidentalis*. Though relatively higher WUE was noticed in Salix viminalis (Lindroth et al. 1996), water availability was identified as the critical factor in short rotation willow forestry. Tuomela (1997) studying the physiological and morphological responses of *Eucalyptus microtheca* provenances suggested that the efficient control of water loss was indicated by high instantaneous WUE. This suggests that EC 1 and EC 4 could be considered as efficient clones, especially under e levels of CO_2 with reference to water utilization. With reference to the intrinsic carboxylation efficiency, clones IFGTB EC 1 and EC 4 recorded the highest CE under e CO_2 levels over the control (Fig. 4b). This ratio varied between 0.001 and 0.030 μmol m^{-2} s^{-1} (μl l^{-1})$^{-1}$. At a given stomatal conductance, lower Ci indicated better mesophyll efficiency and better draw down rate of the substrate CO_2. It has been reported that drought tolerant cultivars of *Morus alba* exhibited greater mesophyll efficiency than the drought sensitive genotypes (Ramanjulu et al. 1998). In the present study, none of the clones showed an increase in the mesophyll efficiency under e CO_2 conditions.

Fig. 2. Pn-Ci curve under open field, ambient CO_2 in OTC and OTC with e CO_2 levels

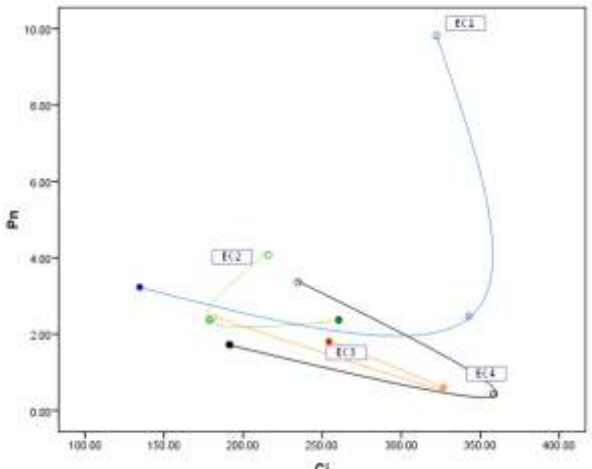
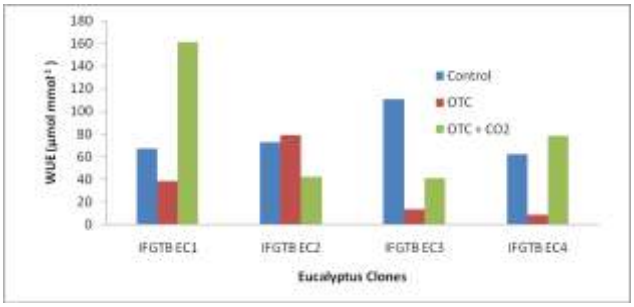


Fig. 3. WUE as influenced by e CO_2 in *Eucalyptus clones*



Many researchers have studied the physiological adaptations of eucalypts. Srivastava (1993) reported that *Eucalyptus* enhanced water holding capacity in the soil. Osorio and Pereira (1993) reported that WUE was significantly increased by water deficit in *E. globulus* clones. Eucalyptus has the inherent capacity for luxury consumption of water when moisture is abundantly available. The high rate of transpiration reported in certain physiological studies on *Eucalyptus* could be an adaptability mechanism operative under adequate soil moisture only (Srivastava et al. 2003).

Biochemical parameters

Leaf total carbohydrate content and the reducing sugar levels in the clones decreased significantly on exposure to e CO_2 levels in the clones EC 2 and EC 3. Clones EC 4 and EC 1 showed significant difference in total soluble sugar content between the CO_2 treatments. Two-way ANOVA showed a significant ($P < 0.05$) interaction between clones and e CO_2 for total carbohydrate content and free reducing sugar levels.

There was also a significant variation in the chlorophyll a: b ratio, both amongst the clones and as a result of e CO_2 (Table 3). There was substantial variation between the clones in the extent and nature of alteration in photosynthetic characteristics. It was observed that though there was a reduction in the photosynthetic rate in response to e CO_2, the trend observed was the same in all four clones. Another observation was that the reduction in photosynthetic rate due to e CO_2 did not significantly impact the carbon gains being made, as plants exposed to e CO_2 had approximately twice the biomass of plants grown at ambient CO_2. This implies that leaves grown at high CO_2 can capture the photons for photosynthesis similar to leaves grown under ambient CO_2 conditions and may be able to overcome physiological forcing (increasing CO_2 levels) with time.

Short term experiments with *Pinus pondrosa, Quercus coccinea, Pinus radiata* and *Populus deltoides* have shown a definite increase in photosynthesis rate up to 40-80% under 600 ppm levels of CO_2 (Couteaux et al. 1992). Devakumar et al. (1998) who studied the effect of e CO_2 concentration on growth and photosynthesis in two clones of *Hevea brasillensis,* have reported findings similar to our results. They reported higher biomass accumulation, leaf area and better growth when compared to ambient air grown plants. Thus adaptive variations are being reported in perennials with altered climatic conditions.

Rising atmospheric CO_2 will directly affect forest plantation productivity by its impact on photosynthetic carbon fixation. While mature trees may not retain more carbon under e CO_2 (Korner et al. 2005), fixation rates in young trees or seedlings grown in e CO_2 have been shown to increase by up to 50%. However, there is expected to be considerable variation between and within species in responses to e CO_2. Seedlings of the sub-tropical species E. grandis have been observed to grow at approximately four times the rate of seedlings grown under atmospheric CO_2 levels (Conroy et al. 1992). Conversely, no response was observed in the arid zone species *E. occidentalis* (Southerton 2007). It appears that fast growing coppice systems could be considerably more productive in e CO_2, and could contribute to slowing the rate of rise in atmospheric CO_2.

In trees, e CO_2 can increase total leaf area (Koch et al. 1986), leaf weight (Brown and Higginbotham 1986; Norby and O'Neill 1989), leaf weight to area ratio (Conroy et al. 1986, Berryman et al. 1993; Pettersson et al. 1993), and branching frequency (Sionit et al. 1985; Samuelson and Seiler 1993). Reports state that e CO_2 enhances photosynthetic rates in tropical and sub-tropical trees. Accordingly, it should also lead to increased carbohydrate and biomass production in these species. At a tropical forest research site in Panama, twice-ambient CO_2 concentrations enhanced foliar sugar concentrations by up to 30% (Wurth et al. 1998), while doubling the foliar concentrations of starch (Lovelock et al. 1998) in a number of tree species. In the eight-month study of Roden et al. (1999), *Eucalyptus pauciflora* seedlings growing at 700 ppm CO_2 displayed seasonal rates of net photosynthesis that were approximately 30% greater than those exhibited by their ambiently grown counterparts. In another eight-month study, Palanisamy (1999) reported

that well-watered *Eucalyptus cladocalyx* seedlings exposed to 800 ppm CO_2 exhibited photosynthetic rates that were 120% higher than those observed in control plants growing at 380 ppm CO_2. Moreover, after a one-month period of water stress, photosynthetic rates of CO_2-enriched seedlings were still 12% greater than rates displayed by ambiently grown water-stressed seedlings.

Because e CO_2 enhances photosynthetic rates in eucalyptus species, this phenomenon should lead to increased biomass production in these rapidly growing trees. In the eight-month experiment of Gleadow et al. (1998), for example, *Eucalyptus cladocalyx* seedlings growing at 800 ppm CO_2 displayed 134 and 98% more biomass than seedlings growing at 400 ppm CO_2 at low and high soil nitrogen concentrations, respectively. Similarly, *Eucalyptus pauciflora* seedlings growing at twice ambient CO_2 concentrations for eight months produced 53% more biomass than control seedlings (Roden et al. 1999). After the first six weeks of the study, the plantlets grown in air of e CO_2 concentration exhibited an average net photosynthetic rate across all media treatments that was 26% greater than that displayed by plantlets grown in air of 400 ppm CO_2. This phenomenon led to a 23% increase in CO_2-enriched plantlet total dry weight across all media treatments. In addition, after the final four weeks of growth in air maintained at 400 ppm CO_2, the plantlets that were previously exposed to air of 1200 ppm CO_2 displayed survival percentages that were 13% greater than those of plantlets previously grown in ambient air.

CONCLUSIONS

The general observation in our study was that clones of *Eucalyptus* selected from a broad genetic base and tested for their stability across different agro-climatic locations responded differently to e CO_2 levels. The biomass, photosynthetic rates and photosynthetic pigments were high at e CO_2 levels. More research is underway to understand the clonal response to increasing CO_2 concentration and the consequent molecular changes occurring towards adaptability to changing CO_2 levels.

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