INTER- AND INTRA-SPECIFIC VARIATIONS OF CASUARINA UNDER ELEVATED CO_2

By

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THESIS

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DECLARATION

I, hereby declare that the thesis entitled **"Inter and intra-specific variations of Casuarina under elevated CO_2"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associate ship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled **"Inter and intra-specific variations of Casuarina under elevated CO_2"** is a record of research work done independently by **Mr. Abhin Sukumar P.** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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EXTERNAL EXAMINER

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SYMBOLS AND ABBREVIATIONS

aCO_2	- Atmospheric carbon dioxide
EC	- Elevated carbon dioxide
ECET	- Elevated Carbon dioxide and Elevated Temperature
CC	- Chamber Control
SFW	- Stem Fresh Weight
RFW	- Root Fresh Weight
LFW	- Leaf Fresh Weight
TFW	- Total Fresh Weight
SDW	- Shoot Dry Weight
RDW	- Root Dry Weight
LDW	- Leaf Dry Weight
TDW	- Total Dry Weight
RSR	- Root-Shoot Ratio
SVI	- Stem Volume Index
RWC	- Relative Water Content

CHAPTER 1. INTRODUCTION

Casuarina is a fast growing species widely planted in the tropics, sub tropics and Mediterranean countries because of their ready adaptability to a variety of environmental conditions and also for their rapid growth performance. India has the richest history of casuarina cultivation and improvement since its introduction during second half of nineteenth century. Originally introduced in the coastal areas for fuel wood needs, its cultivation has gradually extended to all areas in the peninsular region making India the largest casuarina growing country with around half a million hectares of plantations. The major species planted are *Casuarina equisetifolia* and *Casuarina junghuhniana*, have grown only for the past 10 years and rapidly increases the area of cultivation (Krishna kumar *et al*., 2014). The weather requirement for proper growth of the plant includes an absolute maximum and minimum temperature in the range of 35°C-49°C and -4 °C - 18°C and an annual rainfall of 750-4,500 mm. it is best suited in alluvial soil having a considerable proportion of sand and good moisture supply. Also it survives on poorly drained sites.

Climate change usually refers to an unprecedented change in the various weather parameters or variability observed over a long period of time. Because of anthropogenic activities, worldwide (aCO_2) and temperature, both key variables affecting the plant growth, development and function, have changed in the recent past and are predicted to rise in the future (Wang *et al.,*2012). The atmosphere [CO_2] could reach 540 ppm by 2050 and up to 940 ppm by2100, depending on emissions and adherence with global agreements such as the Paris Climate Agreement. Recent forecasts, including increases in aCO_2 and other greenhouse gases, are largely responsible for recent increases in global mean surface temperatures, which increased by 0.6°C from 1990 to 2000 and are projected to increase by another 1.4–5.8°C by 2100. The plant responses to elevated CO_2 are obtained by conducting experiments in growth chambers, controlled environmental chambers, greenhouses, Open Top Chambers (OTC) and Free Air CO_2 Enrichment (FACE). Nowadays, OTC and FACE derived systems are the most frequently used methods to study tree responses to elevated CO_2 under close to natural conditions. Open Top Chambers are extensively used to study the CO_2 response of plants (Rogers *et al.,* 1983). Open Top Chambers are first used to study the effects of pollution and later it adopted for the CO_2 response experiments (Heagle *et al.,* 1973).

To evaluate the response of intra-specific variation in tree productivity to CO_2, Resco de Dios *et al*.,(2016) used meta-analysis. They observed significant intra-specific variation in the photosynthesis CO_2 response, stem biomass, stem volume and height and found that the magnitude of intra-specific variation in response to elevated CO_2 variation was similar to previous inter-specific variation findings. In dry matter accumulation among various clones under elevated CO_2 conditions, greater intra-specific variation is observed and this variation could be explored in all commercially significant tropical tree species and higher tree varieties can be .identified for higher productivity and carbon sequestration potential under projected elevated CO_2 levels for future environmental conditions (Buvaneswaran *et al.,* 2015).

In the future, CO_2-responsive genotypes may be used for assisted gene flow, the managed translocation of genotypes to new locations to promote adaptation to local environmental conditions (Aitken and Whitlock .,2013). In the end, advanced research on intra-specific variation in phenotypic adaptability in agriculture and forestry may be important for identifying genotypes that can increase (Aspinwall *et al.,* 2015).It is important to decide whether there is a major intra-specific variability in the response to elevated CO_2 to make long-term predictions of how plant communities and individual plant species will respond to the increasing concentration of atmospheric CO_2 (Lüscher *et al.,* 2006).

In this study OTC has been employed to investigate the response of *Casuarina equisetifolia* and *Casuarina junghuhniana* clones to elevated CO_2 and natural elevated temperature and also to find out inter and intra specific variations. Finding out the adaptive varieties of Casuarina to the future climatic conditions has a great significance. For agro forestry /afforestation, plants with large root systems that ensure best survival and rapid uptake of water and minerals are more beneficial and this helps both early establishment and maximum growth for reclamation of ecologically degraded sites (Warrier *et al.,* 2013). Casuarina shows significant effects on exposure to elevated CO_2 in terms of shoot characteristics and concentrations of chlorophylls (Warrier *et al.,* 2013). The present study was taken up to understand the responses in the clones of *Casuarina equisetifolia* and *Casuarina junghuhniana* under short term exposure of elevated CO_2 treatments with the following objectives.

1 .To study the morphological and biochemical response of in Casuarina under elevatedCO_2

2. To study about the inter and intra specific variations of Casuarina under elevated CO_2.

CHAPTER 2.REVIEW OF LITREATURE

2.1 CLIMATE CHANGE AND PLANTS RESPONSES

Elevated atmospheric carbon dioxide concentration is a significant reason for climate change (XU *et al*., 2016). Now it is higher than it was at any time in the past 26 million years and is projected to nearly double during this century (Long *et al*., 2004). The global surface temperature is expected to raise 2.6–4.8°C by the end of this century, according to RCP 8.5 (IPCC, 2013). Climate change, including elevated CO_2, rising temperatures and altered precipitation patterns, have markedly affected terrestrial ecosystem structure and function, carbon and water balance, and crop productivity (Lobell *et al*., 2011; Peñuelas *et al*., 2013).

The global environment is changing with rising temperatures and atmospheric carbon dioxide concentrations [CO_2] (Morison and Lawlor, 1999). Global climate observations show the existence of a warming trend that is expected to change the growth and distribution of tree species. Trees are known to be sensitive to rising temperatures (Adams *et al.,* 2009). Looking at the response of trees to global climate change, it is important to look at their response to these environmental factors (Kallarakkal and Roby, 2012). There are sound theoretical reasons for expecting a higher stimulation of net CO_2 assimilation rates by an increase of [CO_2] at higher temperatures.

The CO_2 Fertilization Effect Hypothesis, i.e. rise in atmospheric CO_2, has a beneficial impact on tree growth due to increased availability of carbon, widely tested by CO_2 Enrichment Experiments and empiric dendrochronological research (Huang *et al.,* 2007). Considerable CO2 enriched experiments have shown positive significant physiological and growth responses of trees to CO_2, providing strong evidence to support the direct effect of CO_2 fertilization (increased photosynthesis, water efficiency, above-and below-ground growth) and thus to predict which ecosystems may be most responsive to CO_2 (Huang *etal*.,2007). Compared to plants grown at existing carbon dioxide levels, plants "fertilized" by elevated levels of atmospheric carbon dioxide have increased their photosynthetic rate (Norby *et al*., 1999, Kimball *et al*., 2002, Nowak *et al*., 2004). Plants "fertilized" by elevated atmospheric carbon dioxide levels increased their photosynthetic rate compared to plants grown at existing carbon dioxide levels (Norby *et al*., 1999, Kimball *et al*., 2002, Nowak *et al*., 2004). In addition, carbon dioxide-fertilized plants responded with increased biomass (dry weight), improved water efficiency and increased tolerance of low light levels (Lenart *et al*., 2007).

2.2 MORPHOLOGICAL RESPONSES

Enrichment with carbon dioxide significantly improved both stem height and stem diameter growth in the first and second seasons of growth (Jach and Ceulemans.2000). Poorter (1993) noted that growth in fast-growing species was above that of slow-growing species to elevated CO_2 and that the growth rates in all species were stimulated by 10 per cent. Warrier *et al*., (2013) reported significant shoot fresh weight (SFW) and shoot dry weight (SDW) variations on plants at high CO_2 levels. The combined impact of CO_2 on the fresh and dry weights and the growth period was not significant. In comparison to environmental conditions, under high CO_2 level approximately 40.27 per cent was increased in SFW and 52.91per cent was raised in SDW. Under high CO_2 conditions, the shooting length was increased compared to environmental conditions (Janani *et al*., 2016). The shoots are increasing more often under higher CO_2 conditions as a result of increased root length under higher CO_2 environmental conditions, observed by Pokorny *et al*., (2012).

Lin *et al*., (2000) reported that an increase in atmospheric CO_2 generated in root production on underground plant growth and development typically increases growth rates of root, especially those of the fine rooted ones. In Wullschleger *et al*., 1992 the conclusion was drawn that, due to increased carbon allocation for root growth, plants grown under high CO_2 had an increased surface and root volume. Such an increase of root surface allows plants that grow under high CO_2 to use more water even from deep layers of soil (Reddy *et al*., 2010).Such an increase of root surface allows plants that grow under high CO_2 to use more water even from deep layers of soil (Reddy *et al*., 2010).The conclusion is that CO_2 enrichment favours nitrogen fixation, which increases the nodule mass, and also increases the root length. Based on the documents available to date on the influence of CO_2 enrichment on mycorrhizal workings, carbon allocation to roots could be increased. Higher CO_2 can boost root or root growth and give positive feedback on plant growth (Shinano *et al*., 2007). Increased CO2 exposure of the plants shows that root crops were increasing, longer, thicker and faster (Chaudhuri *et al*., 1990) in many plant species, increasing root length (Norby 1994, Pritchard & Rogers 2000, Bernecchi *et al*., 2000).

Increased uptake of C, for example, could result in a shift in allocation patterns, and thus in root: shoot ratios, resulting in a change in root physiology and growth (Ceulemans *et al*., 1999). However, long term reactions of trees with high CO_2 did not have a significant RSR impact in L. the tulip (Norby *et al*., 1992).. Gleadow *et al*., (1998) observed in *Eucalyptus* seedlings under elevated CO_2 level a stimulated root growth of 33 per cent and higher root: shoot ratio. Wullschleger *et al*., (2002) similarly argued that the root shoot ratio (RSR) or fine-root proliferations were increased by the impact on growth, gas exchange and plant water relations in enriched CO_2 studies. Various trials have shown that increased CO_2 increases the roots to shooting ratio, thereby enhancing the root system's capacity to acquire soil nutrients (Stulen and Den Hertog 1993, Norby 1994, Rogers *et al*., 1994). The total absorption of nitrogen was reported to decrease elevated carbon emissions (Rothstein *et al*. 2000) without effect Bassiri Rad *et al*. 1997), or to rise (Bassiri Rad *et al*., 1996) in the nitrogen absorption rate (Patterson *et al*., 1988).

Generally, elevated CO_2 concentration increases stem biomass (Curtis *et al*., 1998; Saxe *et al*., 1998; Ainsworth and Long, 2005). Norby *et al* (1992) demonstrated that .For 2.5 growing seasons, *Liriodendron tulipifera* trees grown with enriched CO_2 had 27 percent more dry mass than trees grown with ambient CO_2. As plants grow larger, more biomass is being invested in tissue support (Givnish 1988; Konings *et al*. 1989). In comparison to the plants maintained at 350 ppm, short-term CO_2 exposure considerably increased net Assimilation Rate, Dry Matter production, total dry weight (TDW), dry leaf weight (LDW) and specific leaf weight at 26/17 C. A 100, 87, and 68 percent increase of TDW of cotton, anodes and velvet blades was observed as a result of the short-term enrichment in CO_2 (Patterson *et al*., 1988). At the level of the genotype, significant increases in photosynthesis do not translate proportionally into aboveground growth increases of comparable magnitude, where the C allocation should be distributed.

In trees, elevated CO_2 can increase, plant height, total leaf area, leaf weight, leaf thickness, dry weight, total biomass and yield of the plants. (Koch *et al*., 1986). A similar study in Strawberry shows an increase in plant height, leaf area and biomass (Chen *et al*., 1997). Elevated CO_2 often increases total leaf area, leaf weight and leaf weight-to-area ratio (Ceulemans, 1997; Norby *et al*., 1999). Ceulemans *et al.,*(1995) observed that the leaf area was increased8-18 percent in *Populus* clones under CO_2 enrichment which lead to increased leaf area index. Open-top chamber experiments with CO_2 enrichment usually show an increase in leaf area of seedlings and saplings (Norby *et al*., 1999). Tissue *et al.* (1997) reported a 217, 80, 58 and 41per cent increase in leaf area of loblolly pine (*Pinustaeda* L.) growing in elevated CO_2 environment for subsequent four growing seasons respectively when compared to ambient CO_2. Ceulemans *et al.* (1995) observed 8–18per cent leaf area increases for *Populus* clones under CO_2 enrichment.

2.3 BIOCHEMICAL RESPONSE OF PLANTS TO [ECO_2], [ET] AND [ECO_2] + [ET]

2.3.1 Carbohydrate content

Exposure to elevated CO_2 not only caused a significant increase in the soluble sugar and starch content, but also caused an increase in the cellulose content. The total soluble sugar and starch contents per unit LDW in *Arabidopsis thaliana* grown in elevated CO_2 increased by more than 70per cent ($P < 0.001$) and approx. 80per cent ($P < 0.001$), respectively (Teng *etal*., 2006). Inter-specific variations of leaf and root starch concentrations were formed in seedlings of five boreal tree species grown under 370 and 580 (Tjeolker *et al*., 1998). Plants grown in elevated CO_2 usually have increased soluble sugar and starch content (Delucia *et al*., 1995). Warrier *et al* .2013 reported that there is no significant variation in carbohydrate levels in *Casuarina equisetifolia, Tectonagrandis* and *Ailanthus excels* under elevated CO_2. Total carbohydrates, starch, soluble sugars and total nonstructural carbohydrates increased under elevated CO_2 (+23per cent, +50per cent, +8per cent and +39per cent, respectively), but structural carbohydrates decreased significantly (13per cent) (Robinson *et al*., 2012). Total flavonoids (TF), total phenolics (TP), total soluble carbohydrates (TSC), starch and plant biomass increased significantly ($P \leq 0.05$) in all parts of the ginger varieties under elevated CO_2 (800 μmol mol−1). Nonstructural carbohydrates, expressed on a leaf area basis, were higher in midday-sampled leaves of plants grown at elevated CO_2 than those grown at ambient CO_2 regardless of growth temperature (Vu *et al*., 2005). In some studies, with conifers, elevated CO_2 had no effect on carbohydrate concentration (Campagna and Margolis 1989, Chomba *et al*. 1993, Jach and Cealumans (2000), whereas other studies shows an increase in accumulation (Balaguer *et al*., 1995).

2.3.2 Total phenol content

Total phenol content in *Dactylis* and *Bromus* was increased by 15 percent and 87 percent respectively, and there were no significant interactions between CO_2 and genotype observed in either of these two species (castells *etal.,* 2002). Total phenolic content was significantly increased in birch leaf under elevated CO_2 (kuokknen *etal.,* 2003). Castells *et al*. (2002) studied two perennial grasses of *Dactylisglomerata* and *Bromus erectus* under elevated CO_2 level and found that the total phenolic concentration increased by 15per cent and 87per cent respectively, and there were no significant CO_2**x** genotype interactions in these species. Wetzel and Tuchman (2005)grew cattails in opentop chambers and they found green leaf material contained 40.6 per cent of total phenolic content in elevated CO_2 levels compared to ambient conditions.

2.3.3 Chlorophyll content

Chlorophyll a, b and total chlorophyll shows significant increase with elevated CO_2, in *Tectonagrandis* (Warrier *et al*., 2013). In elevated CO_2, Black Spruce from all regions had higher total chlorophyll concentrations than Red Spruce. Ontario spruces maintained the greatest difference, whereas the **NS** spruces shows the least difference. Concentrations of chlorophyll a, chlorophyll b and total carotenoids shows similar responses to elevated CO_2 as described for total chlorophyll concentration, but with different magnitudes. There was a significant (RS > BS) species effect on the chlorophyll a: b ratio, but no significant region or species × region effects were found. (Major *et al*., 2007). The plants grown under elevated CO_2 also maintained greater leaf chlorophyll content and lipid to protein ratio, especially under conditions of water stress (Idso *et al*., 2014). The mean chlorophyll content was increased under elevated CO_2 across the genotypes while there was decline in mean chlorophyll content under elevated temperature condition.

2.3.4 Soluble protein

The spring wheat grown under the elevated CO_2 shows greater protein concentrations but no change in soluble protein in soybean (Chen *et al*., 2004). Major food crops such as soybean, barley, wheat, potato and rice exhibited reduction in protein content under elevated CO_2. The soluble protein content was not significantly affected in beech seedlings grown in ambient CO_2 and supplied with high, sufficient or low nutrient solutions (Polle *et al*., 1997). In this study, five independent methods affirm that CO_2 enrichment inhibits NO^3assimilation in wheat and *Arabidopsis* plants. The predominant form of N available to plants in most environments is NO^3; therefore, CO_2 inhibition of NO^3 assimilation would lead to lower organic N production. Indeed, this could be responsible for the 7.4 to 11per cent decrease in wheat grain protein (15, 16) and the 20per cent decrease in total protein content of A. *thaliana* (Columbia) observed under CO_2 enrichment in FACE (free-air CO_2 enrichment) experiments (Bloom *et al*., 2010). In a five-year experiment with *Populusnigra* L., elevated CO_2 and nitrogen fertilization, alone or in combination, did not affect lignin concentrations in the wood. Soluble phenol and soluble proteins in wood decreased slightly in response to elevated CO_2. Higher nitrogen supply stimulated formation of carbon based secondary compounds and increased protein concentrations (Luo *et al*., 2008)**.**

2.3.5 Reducing sugar content

Elevated CO_2shows increase in the concentration of reducing, non-reducing and total sugar in potato leaves (Allen *et al*., 2014). The reducing and total sugar in the leaves of wheat at elevated CO_2 was greater than plants grown in ambient condition. But sugar content was decreased in sunflower and mung bean under elevated CO_2. The lack of acclimation in the current year needles may be related to sink strength with the rapid expansion of these needles serving as an active sink for the carbon gained. Despite this, current-year needles had twice the concentration of soluble sugars and the total non-structural carbohydrates were more than 25per cent higher than in 1-year-old needles. (Griffin *et al*., 2000). If starch accumulation would have less of a feedback on photosynthesis than the accumulation of soluble sugars, this could perhaps form an explanation for the differential decrease in SLA. However, this is at the moment merely a speculative hypothesis (Poorter and Navas. 2003). Under elevated CO_2, reducing sugars (glucose + fructose) increased by 31 and 39per cent, sucrose by 20 and 99per cent and total soluble sugars by 26 and 59per cent at near-ambient and high growth temperature, respectively (Vu *et al*.,2005). Of the non-structural carbohydrates, only soluble sugar was significantly affected by CO_2 treatments increasing approximately 16per cent over ambient CO_2grown leaves. (Williams *et al*., 2000). The present result suggested that enrichment of *Labisia pumila* under high CO_2 was able to enhance the soluble sugar (Ibrahim and Jafar. 2011).

2.4 INTER-SPECIFIC VARIATION OF PLANTS UNDER ELEVATED CO_2

Significant effects of elevated CO_2 on total biomass have been identified by Tischler *et al*., (2004) for mesquite (*Prosopis glandulosa*) on day 3 and for parkinsonia (Parkinsonia aculeata L.), honey locust (*Gleditsia triacanthos* L.), and huisache (*Acacia farnesiana* (L.) Willd.)on day 8. For a range of tree species grown under field conditions similar increases in dry mass have been observed in response to CO_2 enrichment (Norby *et al*., 1999). It has been found that elevated CO_2 causes greater allocation to root biomass (Dickson *et al*., 1998) and this has been observed for all three species in our study. In tree species, an increase of 15 percent in stem height and 30 -45 percent in biomass under elevated CO_2 was reported (Ceulemans *et al*., 1996). An 8 percent increase in height and biomass (15 percent-30 percent) was observed in hybrid poplars with high CO_2 emissions (Tupker *et al*. 2003).

2.5 INTRA-SPECIFIC VARIATION OF PLANTS UNDER ELEVATED CO_2

The phenotypic (yield) response of cloned genotype (9-14 per species) from 12 different grassland species has been determined in an intra-specific variability in the responses to high CO_2 in Swiss FACE. The answer of genotypes has been studied for three years in plant communities under field conditions. For the genus L, the average effects. Evergreen, L. *Multiflorum*, *Arrhenatherumelatius*, *Festucapratensis*,*Holcuslanatus*, *Dactylisglomerata*, *Trisetumflavescens*, *Rumexobtusifolius*, R. *Ranunculus friesianus*, *acetosa*, *Trifoliumrepens* and *Trifoliumpratense* did not display statistically significant intra-specific heterogeneity in the response to elevated CO_2 (Lüscher et al., 1997). However in response to elevated CO_2 there is still significant variance between species (Norby *et al.* 1999). To test intra-specific variance in tree productivity responses to elevated [CO_2], Rescode Dios *et al.* (2016) used meta-analysis. They observed significant variance in the intra-specific CO_2 response of photosynthesis, stem biomass, stalk volume and height and found that the extent of intra-species variation in response to higher CO_2 was close to previous observations of inter-specific variation (Ceulemans*etal*.,1996). For most of the morphologic observations such as height, leaf area, LDW, SDW and root dry weight, Tupker *et al*., 2003 observed major variations in hybrid poplars (RDW). Both aspen and hybrid poplars displayed clonal variance in morphology, development and physiology, with a comparatively low influence of high CO_2 on these clonal variations for aspen and more clonally specific response of CO_2 populations (Tupker *et al*. 2003) .In RRII 105, the assimilation rates increased by almost 75per cent at high CO_2 levels, whereas GT-I increased by only 14per cent. Likewise, the production of RRII 105 is higher than that of GT-I. It can therefore be concluded that clones with higher carbon assimilation capabilities at higher CO_2 concentrations would perform better under elevated CO_2 conditions, as seen in the case of clone RRII 1055 (Devakumar *et al*., 1998). There were no Clonal differences in plant height in the elevated CO_2 treatment at the end of the second growing season, Beaupré and Robusta plants were 7 and 18per cent taller, respectively, in the elevated CO_2 treatment than in the ambient CO_2 treatment (Ceulemans *et al*.,1996). The CO_2induced responses of clone Beaupré included increased investment in branch and leaf biomass that resulted in a significantly increased leaf area index. The CO_2induced responses of the slow-growing clone Robusta included an increase in height growth and increased investment in branch biomass and total leaf N content; however, these increases were not reflected in a higher LAI. (Ceulemans *et al.*, 1996).

Light saturated concentrations of the photosynthesis under high levels in FACE experiments were recorded by both Ainsworth and Long2005 to be 19 percent higher in FACE experiments at 25°C and below, whereas those above 25°C were upto 30 percent photosynthetic rates. High temperatures can also affect/alter the carbon consumption rates from the increasingly increasing metabolic sinks, thus decreasing accumulation of carbohydrates, which in turn improve photosynthesis upregulation under high CO_2 levels.

2.6 *CASUARINA EQUISETIFOLIA* AND *CASUARINA JUNGUNIAHNA*

After its start in the second half of the 19th century, India has a rich history of casuarina rising and improvement. Introduced originally in the coastal area for fuelwood needs, the cultivation of *Casuarina equisetifolia* and *Casuarina jungsuhniana* has been slowly spreading to the entire region of the peninsular, making India the biggest casuarina growing territory, with about half a million hectares of plants. There was a mistake (Krishna kumar *et al*., 2014). Casuarina is a quickly growing species of light. Excess soil moisture, fire and frost are very susceptible. It is well drained in sandy soil and it grows terribly in hard soil and tolerates no clay. By its heavy root nodulation with nitrifying bacteria, it increases soil fertility. The key end use of a casuarina is paper manufacturing, though traditional uses such as fuelwood, building poles and props for farming crops are still very common. Environmental resources include shelter belts, windbreaks and reclaiming lands impacted by mining and salt. In addition to fuel, wood is commonly used for papermaking, and late is a favoured alternative for the production of biomass electricity. In rural buildings and as construction scaffolds, the straight cylindrical stalks are seen. It is the key species for coastal shelters and windbreaks for agricultural crops defence. It would have more nodes, more branch thickness, broader branch angles, higher growth rate and biomass productivity, and are ideal for windbreaks and thus have a significant role to play in restoring mined areas and improving sites with low nutrient usage (Nicodemus.2014). There was a mistake (Buvaneswaran *et al*., 2014)

CHAPTER 3.MATERIALS AND METHODS

The present research was aimed at examining the inter and intra specific variations of *Casuarina equisetifolia* and *Casuarina junghuhniana* under projected climate variables (elevated CO_2 and temperature). Six clones of each species were used to study the intra-specific variations. These clones have been generated through a systematic tree improvement programme at IFGTB, Coimbatore. *Casuarina equisetifolia* and *Casuarina junghuhniana* clones were exposed to EC (800 ppm) temperature reduced using humidifier, Ambient (400ppm), CC (Chamber Control) natural elevation of temperature due to chamber effect, ECET, and CO_2 concentrations using Automated Open Top Chambers (AOTCs).

3.1 LOCATION AND GEOGRAPHY

The research was conducted on Automated open top chamber facility from 1st August to 28th October 2017 in the, Forest Ecology and Climate Change Division, Institute of Forest Genetics and Tree Breeding at Coimbatore located at 11°59'01.69" N, 76°57'25.32" E and 437 m above mean sea level. Experimental site experiences maximum temperature of 44 ºC and minimum temperature of 36 ºC, average annual rainfall of 315 mm and 77per cent relative humidity.

3.2 EXPERIMENTAL PLANT MATERIAL

Clones of *Casuarina junghuhniana* used for the experiment were

- CJ-12
- C-J6
- C-J8
- CJ-18
- CJ-17
- MTP-27

Clones of *Casuarina equisetifolia* used for the experiment were

- TCR -12-02-03
- TCR- 02-01-01
- TCR- 06-01-01

- CE- 243
- CE-224
- CE-21

Phylloclade's of the clones were treated with bavistin to remove all fungal infections and later dipped in 2000ppm IBA solution. They were placed in hycopots (60cc) in coir pith.

Plate 1: Casuarina clones transplanted to polybags

Rooted cuttings were then transferred to the polybags of size 10×25 cm containing standard nursery potting mixture of soil, sand and compost in the ratio 1:2:1. Then clones were hardened for three month after which, the clones were taken for the experiment.

3.3 EXPERIMENTAL SETUP

The experiment was carried out in three automated open top chambers having a size of 3x3x3m constructed with poly vinyl chloride sheets with 90per cent sunlight transmittance, to study the inter and intra-specific variation of *Casuarina* under elevated CO_2. In the experiment, 6 clones of two Casuarina species with 6 replications were transplanted and observed in ambient condition and inside the open top chamber.

Design: Factorial CRD

Treatments

1. Ambient CO_2 (open condition) -Ambient

2. Ambient CO_2 (Chamber Control) - CC

3. 800 ppm CO_2 (Temperature reduced to ambient) - EC

4. 800 ppm CO_2 (no temperature control) - ECET

Plate 2: An Overview of Automated Open Top Chambers facility installed at Forest Ecology and Climate Change Division, IFGTB, Coimbatore

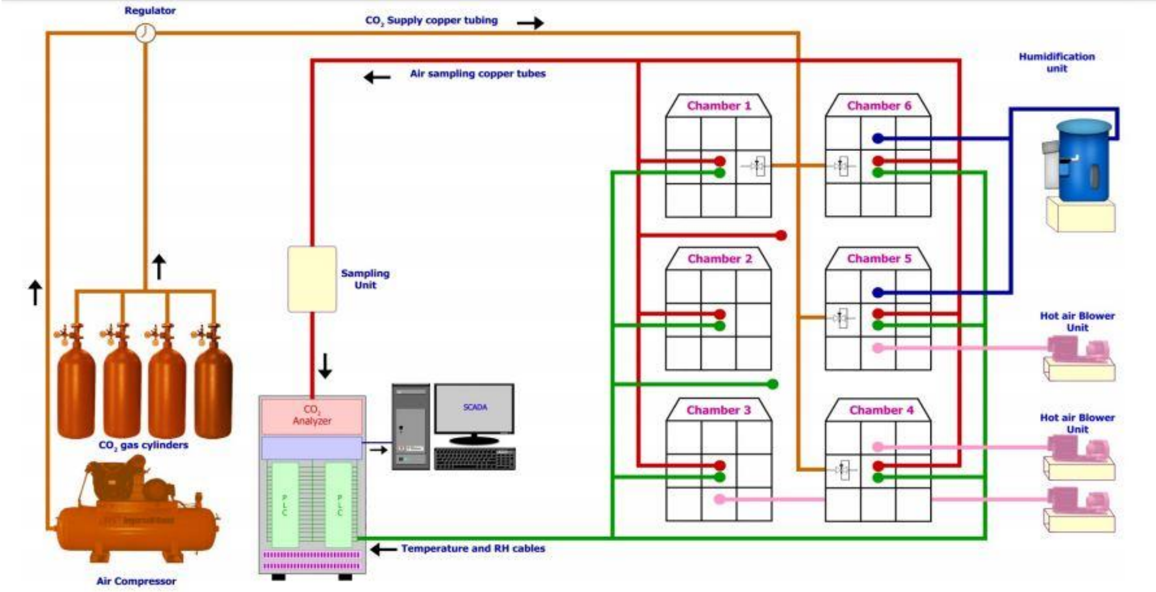

Plate 3: Schematic diagram of Automated Open Top Chambers and the conditions in Silviculture Nursery, IFGTB

3.3.1 Structure of AOTCs

Automated Open Top Chambers (AOTCs) are generally used for exposing plants to elevated levels of CO_2 and other gases besides simulated humidity and temperature. The OTCs are transparent chambers in which CO_2 is pumped to the bottom to maintain the desired levels. The chambers are cubical structures of 3×3×3 m dimension, fabricated with galvanized iron pipe frames. The structures were covered with UV protected polyvinyl chloride sheet of 120 µ thickness in order to have a transmittance of more than 90per cent of ambient radiation. The upper portion of the chamber was kept open to maintain near-natural conditions. AOTCs elevated CO_2 condition was achieved by injecting CO_2 gas (Food grade; Gee Gee Gas Service, Coimbatore, India) at the bottom of the chamber from pressurized cylinders through valves. Temperature and humidity of the AOTC were measured with the inbuilt thermistor and capacitance sensors. In order to record, display and control the actual and desired CO_2 levels, relative humidity and temperature at inside and outside of the each AOTC, data logger software called Supervisory Control and Data Acquisition (SCADA) was used.

The temperature inside the chamber was increased by 2-4 °C above the ambient environment. Treatments were imposed 90 days after establishing the experimental plant materials. It was kept inside Automated Open Top Chambers with specific distance apart from each other and from the chamber walls. Clones were arranged in a randomized design to avoid effects of within-chamber variation in temperature, light and CO_2. Plants were watered daily throughout the experimental period and grown with day/night temperature regimes of prevailing weather conditions of experimental site.

3.4 MORPHOLOGICAL AND PHYSIOLOGICAL OBSERVATIONS

3.4.1 Shoot length

The plant height was measured from the base of shoot to the tip of the shoot using measuring scale for initial and final values and was expressed in cm.

3.4.2 Collar diameter

It was measured in the base of the stem at the root collar region for initial and final values and was expressed in mm.

3.4.3 Needle length

The needle length was measured in each plant with average of 6 needles for initial and final values and the mean value was expressed in cm.

3.4.4 Needle diameter

The needle diameter was measured in each plant with average of 6 needles for initial and final values and the mean value was expressed in mm.

3.4.5 Needle area

The needle area was calculated by using the formula $2\pi rh$.

3.4.6 Root length

At the end of experiment, the root length was measured from collar region to the tip of root and the values were expressed in cm.

3.4.7 Initial biomass production

A day before the start of the treatment (i.e. on 1st of August 2017), three representative CO_2 ramets of each clone were sampled and washed carefully without any damage to roots. Then theshoot, root and leaveswere separated and fresh weight was taken after which, it was oven dried at 60±1°Cfor 3days in order to obtain the initial dry biomass.

3.4.8 Final biomass production

At the end of experiment, three representative ramets of each clone from each treatment were sampled for the measurements of SFW, root fresh weight (RFW) and leaf fresh weight (LFW) by using weighing balance. After measuring fresh weight, the samples were oven dried at 60±1°C and was kept inside the hot air oven for 3days to reach its constant value. Dry weights of roots, leaves and stem were added to obtain the total biomass of the plants under different treatments.

From the observations, the following parameters were derived.

3.4.9 Root/Shoot Ratio (RSR)

The RSR was calculated as per the formula given below,

RSR=Root length (cm)/Shoot length (cm)

3.4.10 Stem Volume Index (SVI)

SVI was calculated by using the formula described by Hatchell, (1985) and Manavalan (1990)

Stem Volume Index = Diameter (mm)^2 x Height

3.4.11 Relative Water Content (RWC)

After 85 days of CO_2 exposure the leaflet samples were collected and fresh weight was recorded using analytical scale with precision of 0.001. Then the samples were left for floating on distilled water in petridishes, for four hours and the turgid weight was recorded. After that, the leaf tissues were dried in hot air oven at 65 ºC for 24 hours and dry weight was measured. The RWC is calculated according to the following formula by Barrs and Weatherley (1962) and was expressed as percentage, RWC (per cent) = (Fresh weight – dry weight)/ (Turgid weight – dry weight) ×100

3.5 BIOCHEMICAL ANALYSIS

To study the response of elevated CO_2 treatment on biochemical factors the samples were estimated for the following parameters at the end of the experiment. Fresh needles were collected for biochemical experiments.

Three replications were considered for each parameter and the values were recorded for quantification of biochemical in *Casuarina.*

3.5.1. Chlorophyll estimation (Arnon, 1949)

Principle

Chlorophyll extraction was done using 80per cent acetone and the absorption was read at 663 nm (for chlorophyll a) and 645 nm (for chlorophyll b) in a spectrophotometer. Using absorption coefficients, the amount of chlorophyll was calculated and expressed in terms of mg per gram of tissue.

Materials

Dilute analytical grade acetone to 80per cent pre cooled acetone.

Procedure

Sample Preparation

- 0.100 g of sample was weighed into a clean mortar.
- The tissue was ground well into fine pulp with 5 ml of 80 per cent acetone.
- The mixture was centrifuged at 5000 rpm for five minutes and the supernatant was collected in a 10 ml clean volumetric flask.
- Repeat the above procedure until the residue becomes colorless.
- The mortar and pestle were washed thoroughly with 80 per cent acetone and was collected in the same tube. The absorbance of the solution was read at 645 and 663 nm against the solvent 80 per cent acetone as blank.

Calculation

The amount of chlorophyll present in the extract was calculated using the following equations:

Mg total chlorophyll = $20.2(A_{645}) + 8.02(A_{663)} \times V \div 1000 \times W$

Where, A=Absorbance at specific wavelengths; V=Final volume of chlorophyll extract in 80 per cent acetone; and W=Fresh weight of tissue extracted.

3.5.2 Carbohydrate estimation by Anthrone method (Hedge and Hofreiter, 1962)

Principle

Carbohydrates are first hydrolyzed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose becomes dehydrated to hydroxymethyl furfural. This compound forms with anthrone and green colored product with an absorption maximum at 630 nm.

Materials

- 2.5 N Hydrochloric acid
- Anthrone reagent – Dissolve 200 mg anthrone in 100 ml of ice cold 95 per cent H2SO4. (Prepared fresh before use).
- Stock standard – Dissolve 100 mg of glucose in 100 ml of distilled water.
- Working standard – 10 ml of stock diluted to 100 ml with distilled water. A few drops of toluene were added and it was stored in refrigerator.

Procedure

Sample preparation

- 0.100 g of the sample was weighed in a boiling tube.
- The sample was hydrolyzed by keeping it in a boiling water bath for half an hour with 5 ml of 2.5 N HCl and then cooled at room temperature.

- The boiled sample was then neutralized with solid sodium carbonate until the effervescence ceases.
- The volume was made up to 5 ml and then centrifuged.
- The supernatant was collected for further analysis.

Estimation

- 100 µl aliquots were taken for analysis.
- Meanwhile, a series of standards were run using glucose (0-100µg).
- 100 µl of the sample extract was taken in a separate test tube.
- The volume was made upto 1 ml in all the test tubes and 1 ml of water served as the blank.
- 4 ml of anthrone reagent was added (before adding anthrone reagent cool the content with ice) to all the test tubes.
- The contents were heated for eight minutes in a boiling water bath.
- Then it was cooled rapidly and the green to dark green color was read at 630 nm
- A standard graph was plotted by using concentration of the standard on the X-axis versus absorbance on the Y-axis.
- From the graph, the amount of carbohydrate present in the sample was calculated and expressed as 'percent per sample'.

3.5.3 Reducing sugar estimation by Dinitrosalicyclic acid method: (Miller, 1972)

Principle

This method is to test the presence of free carbonyl group (C=O), also called as reducing sugars. It involves the oxidation of the aldehyde functional group present in glucose and ketone group present in fructose. Simultaneously, 3,5-Di-Nitro Salicylic acid (DNS) is reduced to 3-amino, 5-nitrosalicyclic acid under alkaline conditions.

Materials

- Di-nitro salicylic acid reagent (DNS reagent).
- 100 ml of 1 per cent NaOH was prepared and it was dissolved by stirring with 1 g dinitrosalicyclic acid, 200 mg crystalline phenol, and 50 mg sodium sulphite and stored at 4 ºC. Usually, prepared fresh before use.
- 40 per cent Rochelle salt solution (Potassium sodium tartarate).

- Stock standard – Dissolve 100 mg of glucose in 100 ml of distilled water.
- Working standard – 10 ml of stock diluted to 100 ml with distilled water.

Procedure

Preparation of sample

- 0.100 gm of sample was weighed and the sugars were extracted with 1.5 ml of hot 80 per cent ethanol in twice.
- The supernatant was collected and evaporated by keeping it on a water bath at 80^{o}C.
- The sugars were dissolved by adding water and made up to 5 ml.

Estimation

- 100 µl of the extract was pipetted out to the test tubes and the volume was equalized to 1 ml with water in all the tubes.
- 3 ml of DNS reagent was added.
- Then the contents were heated in a boiling water bath for five minutes.
- When the tubes still in warm, add 1 ml of Rochelle salt solution.
- Then it is cooled and the intensity of dark red color was read at 510 nm.
- Reagent blank and a series of standards were run using glucose (0-100 µg) and the graph was plotted using absorbance versus concentration. The reducing sugar concentration was calculated in terms of 'percent of sample'.

3.5.4 Protein estimation (Lowry et al., 1951)

Principle

The reduction of the phosphomolybdinic-phosphotunstic components in the Folin-Ciocalteau reagent by the presence of amino acids, tyrosine and tryptophan and protein leads to blue color development in biuret reaction. Here the protein with the alkaline cupric tartarate was measured by Lowry's method.

Materials

- Reagent A-2 per cent Sodium carbonate in 0.1 N Sodium hydroxide
- Reagent B-0.5 per cent Copper Sulphate in 1 per cent potassium sodium tartate

- Reagent C-Mix 50 ml of Reagent A and 1 ml of Reagent B prior to use
- Reagent D-Folin-Ciocalteau reagent

Protein stock standard

Fifty mg of bovine serum albumin (Fraction V) was dissolved in distilled water and made up to 50 ml in a standard flask. Working standard 10 ml of stock solution was diluted to 50 ml with distilled water in a standard flask. (1 ml of this solution contains 200 µg of proteins).

Procedure

Preparation of extract

0.100 g of the leaf sample was weighed and ground well with pestle and mortar in 2 ml of the phosphate buffer. The mixture was centrifuged and the supernatant was used for protein extraction.

Estimation

- 100 µl of the sample extract was taken in a test tube.
- The volume was made up to 1 ml in all the test tubes. The blank served as 1 ml of water.
- 5 ml of reagent C was added to each tube including the blank, mixed well and incubated for 10 min.
- 0.5 ml of reagent D was then added, mixed well and incubated at room temperature in dark for 30 min.
- A series of standards were run using glucose (0-100µg)
- The blue color developed was read at 660 nm.
- The protein content was calculated from the standard graph and expressed as mg per g of sample.

3.5.5 Phenol estimation by Folin-Ciocalteau method (Malik and singh, 1980)

Principle

Phenol reacts with phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium and produce blue colored complex (molybdenum blue).

Materials

- 80 per cent ethanol.
- Folin-Ciocalteau reagent.
- 20 per cent Sodium carbonate (Na2CO3).
- Stock standard-100 mg Catechol in 100 ml water
- Dilute 10 times for working standard from stock solution.

Procedure

Sample preparation

- 0.100 g of sample was weighed and ground in a mortar and pestle with 10 times volume of 80 per cent ethanol.
- The homogenate was centrifuged at 10,000 rpm for 20 min. and the supernatant was collected. The residue was re-extracted by centrifuging with five times the volume of 90 per cent methanol to pool the supernatant.

Estimation

- 100 µl of extract was taken and the volume was made upto 3 ml with distilled water and blank was set with 3 ml water.
- 0.5 ml of Folin-Ciocalteau reagent was added.
- After 3 min, 20per cent Na2CO3 solution was added to each tube and mixed thoroughly.
- A series of standards were run using catechol standard (0-100µg).
- The tubes were placed in boiling water for exactly 1 min. and cooled. The absorbance was measured at 650 nm against reagent blank.
- The standard graph was drawn using absorbance versus concentration to determine the amount of phenol in the samples in terms of mg per gram of sample.

3.6 DATA ANALYSIS

The morphological, and biochemical responses under elevated CO_2 levels with replications were analyzed statistically by applying Software package (SPSS ver. 20, IBM Corporation 1989, 2011, US).

CHAPTER 4.RESULT AND DISCUSSION

4.1 INTER – SPECIFIC VARIATIONS AFTER THREE MONTHS OF STUDY

4.1.1 Morphological adaptive variations between species

Global climate change is operated by the rapid increase of Green House Gases (GHGs) and it has become a major driving force in global atmosphere. Carbon dioxide is one of the most important GHG's due to its exponential rise in concentration in the atmosphere. Consequently, there are changes in global climate which may in turn influence the growth and morphology of the industrially important tree species in the tropics. Moreover, the elevated CO_2 concentrations have a significant impact on plant growth, productivity and species composition in agricultural, forest and natural ecosystems (Kirkham, 2011). Observations on such changes in morphological and biochemical responses under elevated CO_2 levels will provide information's to plant responses to various atmospheric conditions.

Table 1: Root number, Root length, Shoot length and Collar diameter in *Casuarina junghuhniana and Casuarina equisetifolia* for different treatments (Ambient, CC EC, and ECET)

Species	Treatment	Root Number	Root Length(cm)	Shoot Length(cm)	Collar Diameter(mm)
CJ	Ambient	7.09	20.84	25.82	4.06
	CC	6.17	20.61	25.02	3.51
	EC	6.14	21.25	28.38	3.82
	ECET	5.97	21.97	27.68	3.45
CE	Ambient	6.56	19.07	19.90	3.18
	CC	6.72	18.54	23.01	3.56
	EC	7.72	18.54	22.03	3.18
	ECET	7.50	18.49	21.81	3.36

In *Casuarina junghuhniana* it was noted that in, the ambient plants had higher root number (7.09) than CC (6. 17) plants. This is due to the stress induced due to the temperature. Butin *Casuarina equisetifolia,* it was observed that the highest number of roots were seen in EC plants (7.09) (due to CO_2 fertilization) and ECET plants have a slighter number of roots more than CC as the negative effects of the stress were slightly reduced due to CO_2 fertilization. However, this data is statistically non-significant.

In *Casuarina junghuhniana*, the ambient plants had higher root length (20.84c.m) than CC (20.61c.m) Plants. Plants under ECET treatment show higher the root length but in *Casuarina equisetifolia,* it was observed that the root length was seen in ambient plants (19.07). Elevated CO_2 increased total root length by 122 per cent in scots pine seedlings as reported by Janssens *et al*. (1998).

Although the longest combined root and shoot length belongs to the ECET and EC plants, than ambient in *Casuarina junghuhniana* but *Casuarina equisetifolia* shows higher shoot length in CC. The data of shoot length is statistically significant however the root length data is not statistically significant. Collar diameter was the highest in case of ambient plants in *Casuarina junghuhniana* but *Casuarina equisetifolia* the CC plants higher collar diameter. Under EC conditions however, the collar diameter decreased generally (as a higher growth in length was observed) with the EC plants having higher collar diameter than ECET plants as the former was not under heat stress.

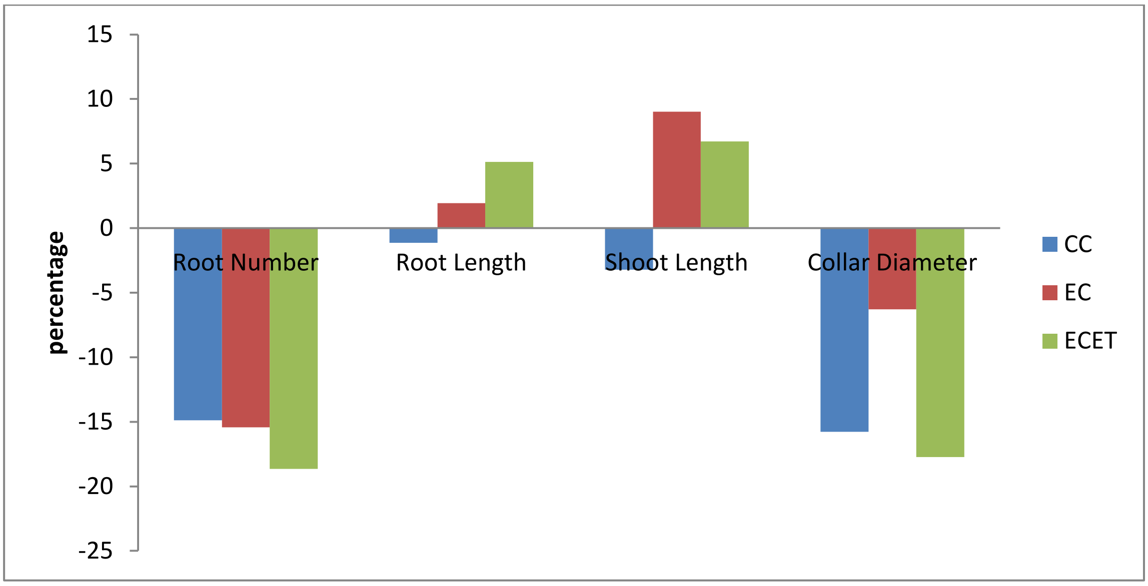

Figure 1: Increase or decrease in growth parameters (root number, root length, shoot length, collar diameter) in clones of *Casuarina junghuhniana* for different treatments (CC, EC and ECET) as percentage over that of ambient condition.

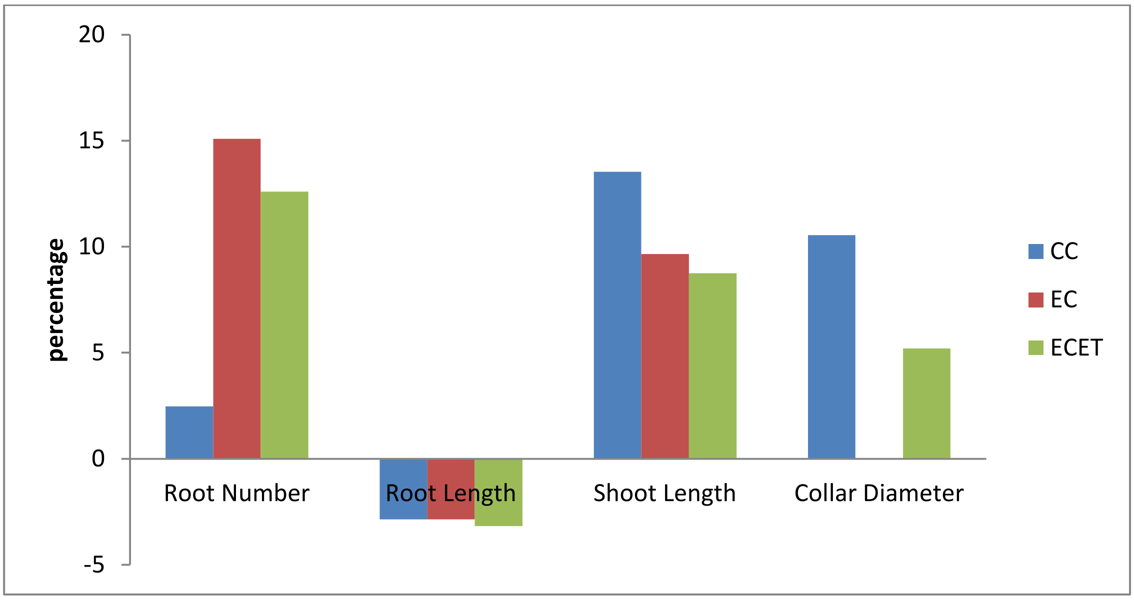

Figure 2: Increase or decrease in growth parameters (root number, root length, shoot length and collar diameter) in clones of *Casuarina equisetifolia* for different treatments (CC, EC, and ECET) as percentage over that of ambient condition.

Table 2: Needle Length, leaf area, stem volume Index in *Casuarina junghuhniana and Casuarina equisetifolia* for different treatments (Ambient, CC, EC, and ECET)

Species	Treatment	Needle Length(cm)	Needle Diameter(mm)	Leaf Area(mm^2)	Stem Volume Index (mm^3)
CJ	Ambient	17.11	0.07	38.2	4.20
	CC	17.32	0.07	35.2	3.12
	EC	18.11	0.07	39.5	4.40
	ECET	16.07	0.07	37.8	3.32
CE	Ambient	13.51	0.07	27.2	2.19
	CC	15.24	0.08	36.0	2.99
	EC	15.83	0.07	35.0	2.57
	ECET	16.13	0.07	35.8	3.04

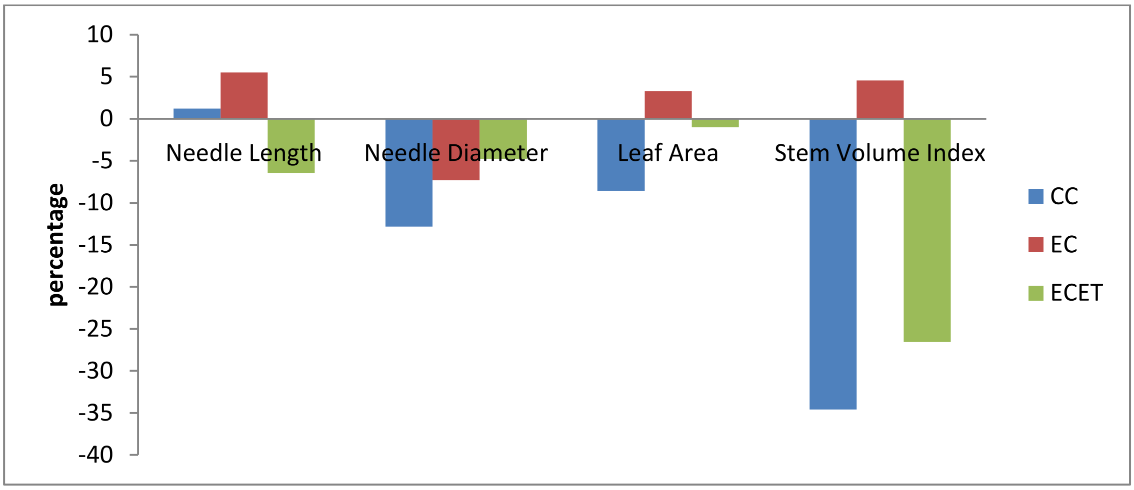

Figure 3: Increase or decrease in growth parameters (Needle Length, leaf area, stem volume index) in clones of *Casuarina junghuhniana* for different treatments (CC, EC, ECET) as percentage over that of ambient condition.

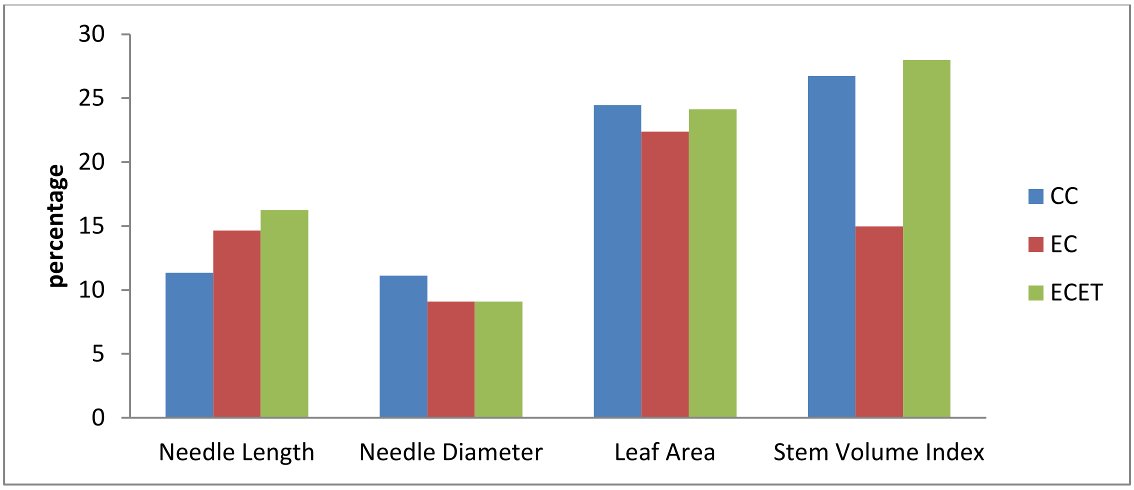

Figure 4: Increase or decrease in growth parameters (Needle Length, leaf area, stem volume index) in clones of *Casuarina equisetifolia* for different treatments (CC, EC, ECET) as percentage over that of ambientcondition.

Increasing atmospheric CO_2 significantly increased the final plant biomass, aboveground biomass, leaf area and belowground biomass (Obrist and Arnone, 2003). Increased root growth contributed to root biomass and RDW under elevated atmospheric CO_2 regardless of species or study conditions (Rogers*et al.*, 1996). Roots often exhibited the greatest relative dry weight gain among plant organs (Norby *et al.*, 1992) even more than aboveground biomass or leaf area production.

In case of needle length, the plants which belonged to EC shows the highest needle length as these plants were under the influence of CO_2 fertilization and a reduced length of needle was observed in the ECET plants as heat stress was present in *Casuarina junghuhniana.*

However, CC plants hadlower needle length than EC and ECET in *Casuarina equisetifolia* due to heat stress. Leaf needle diameter, however was almost same for all the four categories. Both needle length and needle diameters were statistically non-significant.

In Casuarina junghuhniana, EC plants had higher leaf area than normal plants, with ECET plants having lesser leaf area than EC plants, due to the stress imposed on them All the above parameters are statistically significant.

Table 3: Total fresh weight, Total dry weight, Fresh weight root allocation, Fresh weight shoot allocation, and Fresh weight leaf allocation in*Casuarina junghuhniana and Casuarina equisetifolia* for different treatments (Ambient, CC, EC, and ECET)

Species	Treatment	Fresh Weight-Root (g)	Fresh Weight-Shoot (g)	Fresh Weight Leaf (g)	Total Fresh Weight (g)
CJ	Amb	2.543	1.34	3.96	7.81
	CC	1.67	1.14	3.16	5.95
	EC	2.05	1.17	3.86	7.05
	ECET	1.65	1.12	3.73	6.46
CE	Amb	1.95	0.78	2.52	5.25
	CC	1.55	0.95	2.86	5.35
	EC	2.07	0.96	2.88	5.90
	ECET	1.99	1.02	3.19	6.18

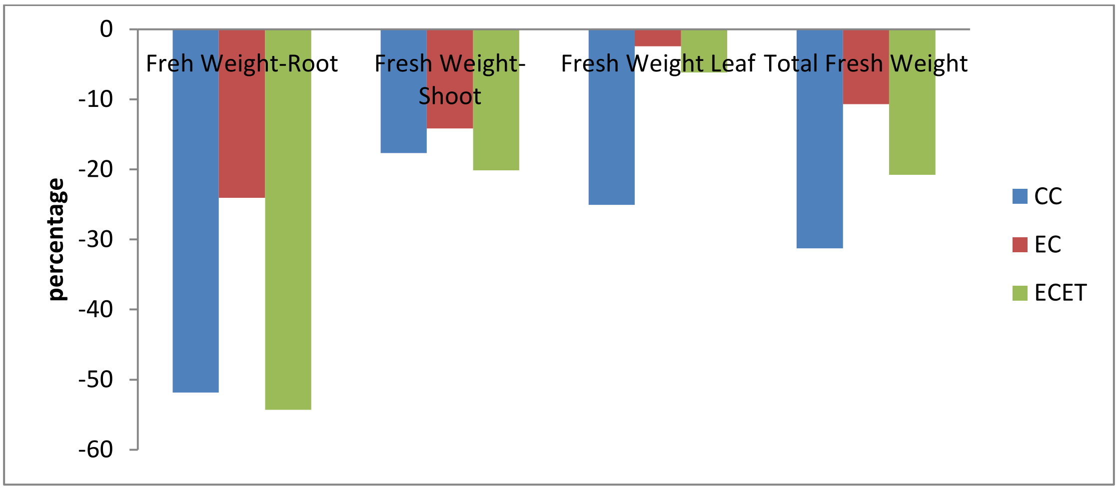

Figure5: Increase or decrease in Biomass (fresh weight) of the *Casuarina junghuhniana* for different treatments (CC, EC, ECET) as percentage over that of ambient

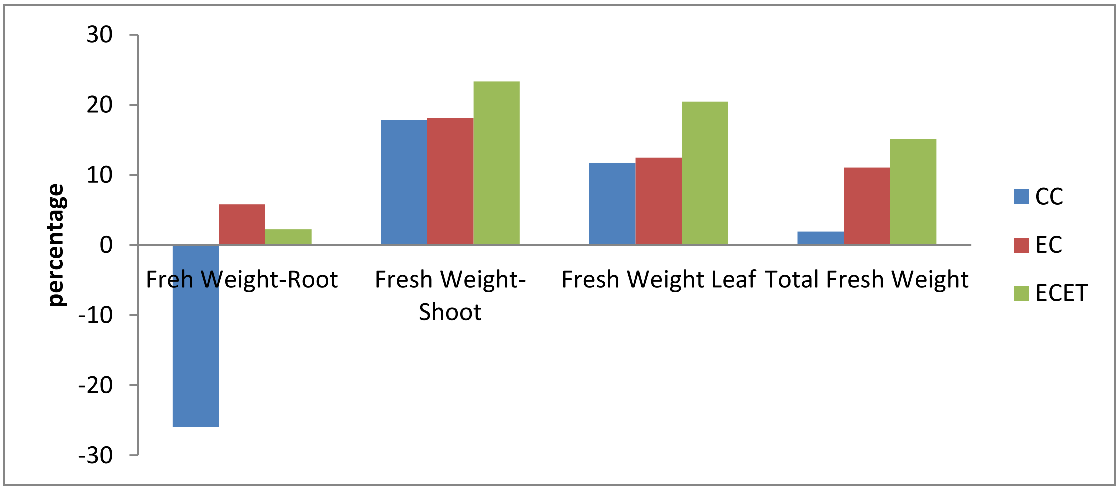

Figure 6: Increase or decrease in biomass (fresh weight) of the *Casuarina equisetifolia* for different treatments (CC, EC, ECET) as percentage over that of ambient

As the plants were not under stress, ambient and EC plants shows higher TFW than their counter-parts, which were under heat stress as higher amount of water transpired from these plants. This is especially true when the TDW and the difference between the TFW and TDW (the water content) were to factor in. A majority of fresh weightwas allocated to leaves in all the four treatments.

However, the root allocation was higher for the heat stressed plants which signifies that, under stressed conditions plants tend to grow more root, so as to get more water from soil. TDW allocation also shows similar properties with the majority of weight being allocated to the leaves and stressed plant roots having more root allocation than their counter parts. A notable exception was the TDW of roots of EC plants which shows similar root allocation as that of EC ET.

Table 4: Root dry weight allocation, Shoot dry weight allocation, Leaf dry weight allocation, MC in *Casuarina junghuhniana and Casuarina equisetifolia* for different treatments (Ambient, CC, EC, ECET)

Species	Treatment	Dry weight-root (g)	Dry weight-shoot (g)	Dry weight-leaf (g)	Total dry weight (g)	MC (per cent)
CJ	Ambient	1.10	0.67	1.59	3.36	57.16
	CC	0.74	0.59	1.25	2.58	56.76
	EC	0.88	0.63	1.53	3.03	57.63
	ECET	0.79	0.59	1.47	2.83	55.92
CE	Ambient	0.69	0.38	1.05	2.11	59.72
	CC	0.69	0.46	1.04	2.18	59.83
	EC	0.77	0.44	1.03	2.23	61.83
	ECET	0.73	0.48	1.14	2.35	62.00

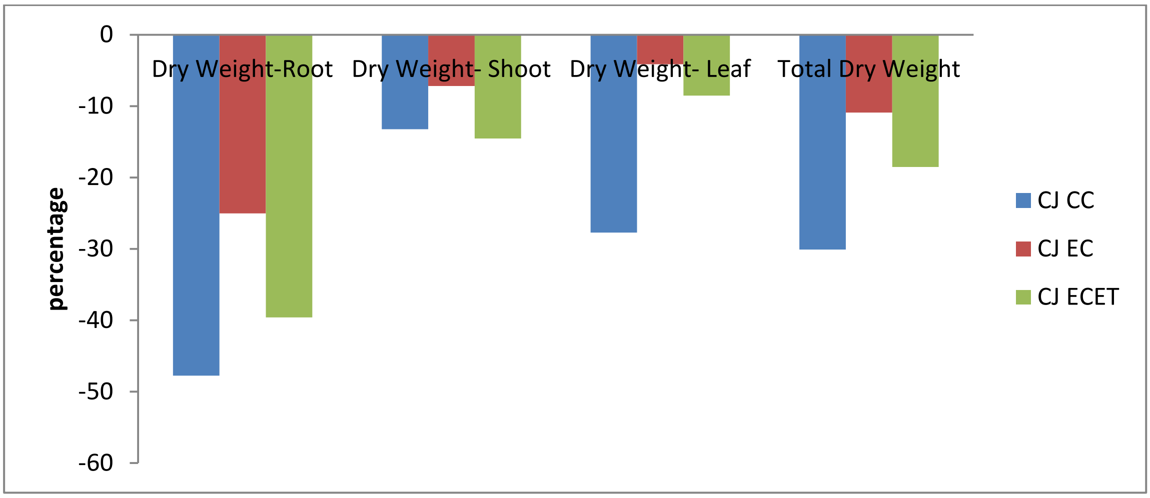

Figure 7: Increase or decrease in biomass (dry weight) of the *Casuarina junghuhniana* for different treatments (CC, EC, ECET) as percentage over that of ambient condition.

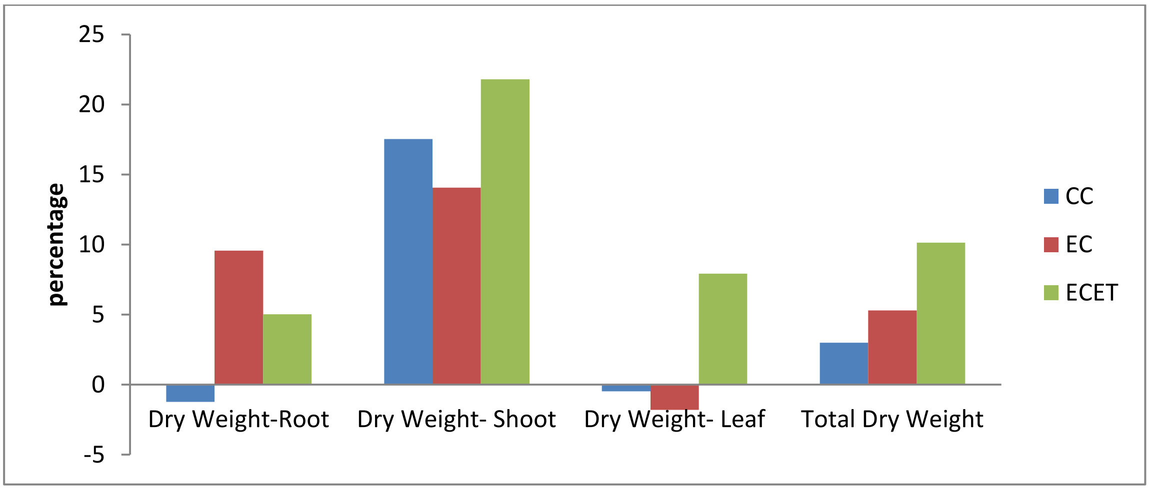

Figure 8: Increase or decrease in Biomass (dry weight) of the *Casuarina equisetifolia* for different treatments (CC, EC, ECET) as percentage over that of ambient condition.

The Value of MC was alsothe highest among the non-heat stress plants and the MC values of heat stressed plants were relatively lower than their respective non stress counterparts. Except the TFW shoot allocation, TDW shoot allocation and TDW leaf allocation, all the other biomass factors are statistically significant. In the present study dry matter accumulation was higher in plants grown under elevated CO_2 than in ambient conditions. Cao *et al.,* (2008) reported that *Gossipiumhirsutum* and the above ground biomass increased under 600ppm CO_2 level.

Luvac *et al.*, (2003) revealed that the CO_2 enrichment increases the below ground biomass allocation in the poplus species and the standing root biomass enhanced by 47-76per cent. Moreover the growth of minjiang firshows significantly positive responses to elevated CO_2withhighervalues of total biomass than in the controlled conditions. (Hou *et al*., 2011).

Ghasemzadeh and Jaafar (2011) reported that, when two varieties of *Zingiherofficinale* were exposed to different concentrations (400 and 800 ppm) it resulted in increased total plant biomass over the ambient conditions.

Mohamed (2013) reported that there was no significant intra specific difference in drymatter production in the tropical dry land species of neem. But when the plants were exposed toincrease atmospheric CO_2 the final plant biomass, above ground biomass and below ground biomass was significantly increased in the tree species. (Madhu and Hatifield, 2013). Similarly, the shoot biomass was approximately 35per cent greater for creeping bentgrass plants grown under elevated CO_2, compared to plants maintained under ambient conditions. The root biomass increased by 37per cent due to elevated CO_2 (Burgess and Huang,2014).

Wang *et al*. (2015) studied the responses of rice production under elevated $CO_2$and reported that there was significant stimulation in above ground biomass (28 percent) and below ground biomass of rice (42 percent). Similar findings of root biomass have been reported by several authors and it was increased by 55 percent in *P.silvestris* (Jach e*t al*., 2000), 32 percent in Pinustaeda (Jackson *et al*., 2009).

4.1.2 Biochemical adaptive variations between species

Table5: Proteins, phenols, carbohydrates, reducing sugar in Casuarina for different treatments (Ambient, CC, EC, ECET)

Species	Treatment	Proteins (Mg/g)	Phenols (Mg/g)	Carbohydrates (Mg/g)	Reducing Sugars (Mg/g)
CJ	Ambient	84.53	6.72	62.60	386.03
	CC	67.82	4.43	61.32	405.96
	EC	82.29	4.55	91.47	386.52
	ECET	64.74	4.37	75.56	409.72
CE	Ambient	104.71	7.66	57.38	423.01
	CC	84.99	4.93	49.11	414.11
	EC	90.66	6.63	78.55	393.16
	ECET	86.57	6.58	60.37	411.81

The amount of protein presents in the ambient and EC were relatively higher than CC and EC ET which were subjected to heat stress. This can be attributed to the decrease in the amount of heat sensitive proteins when subjected to heat stress. However, further studies are to be held in protein profile to determine the same. Meta-analysis done to examine the effect of [ECO_2] on the protein concentration of major food crops found that soybean, barley, wheat, potato, and rice reported that each crop had lower protein concentrations when grown at [ECO_2] (540–958ppm) compared with ambient (315–400ppm) CO_2. In rice, wheat and barley protein content reduced by 10-15percent of the value at ambient CO_2 and tuber protein concentration reduced by 14 percent in potato. But in soybean a small statistically significant reduction of protein concentration was observed (Taub *et al.,* 2008). [ET] causes reduction of protein concentration in leaves. Sunoj (2013) reported that [ET] (2^o C) significantly decreased the protein content about 27 percent in coconut seedlings and similar result was reported by Nareshkumar *et al.* (2007) in coconut. In a study, Dubey (2005) found that temperature above 40°C altered the protein metabolism and protein denaturation in many plants. It was also reported that high temperature reduced the concentration of protein in mulberry (Chaitanya *et al*., 2001), BT cotton (Chen *et al*., 2005) and alfalfa (Erice *et al.,* 2007).

Phenols were highest in ambient plants as heat stress reduced the phenol content of the CC plants. EC was also a reducer of phenol content as evident in EC and EC ET. Among elevated CO_2 plants, the heat stress further reduced the phenolic content. Sunoj (2013) found that the concentration of total phenols in coconut leaves was significantly decreased [ECO_2] and a slight increase was observed in CC conditions after 12 months of exposure. It was also found that there was an inverse relationship between the increase in leaf total phenol concentration and increase in biomass of these pine trees (Penuelas *et al*., 1996).

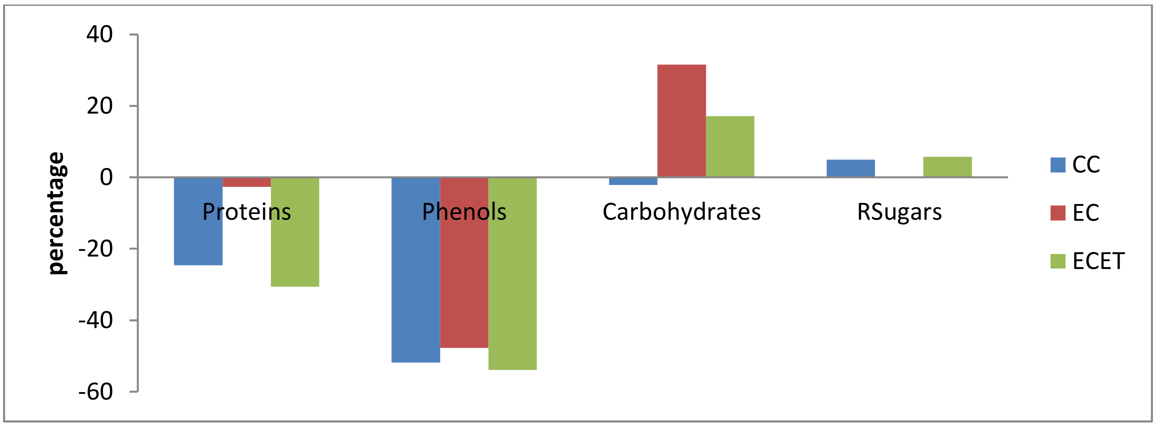

Figure 9: Increase or decrease in proteins, phenol, carbohydrate and reducing sugar of *Casuarina junghuhniana* clones for different treatments (CC, EC and ECET) as percentage over that of ambient condition.

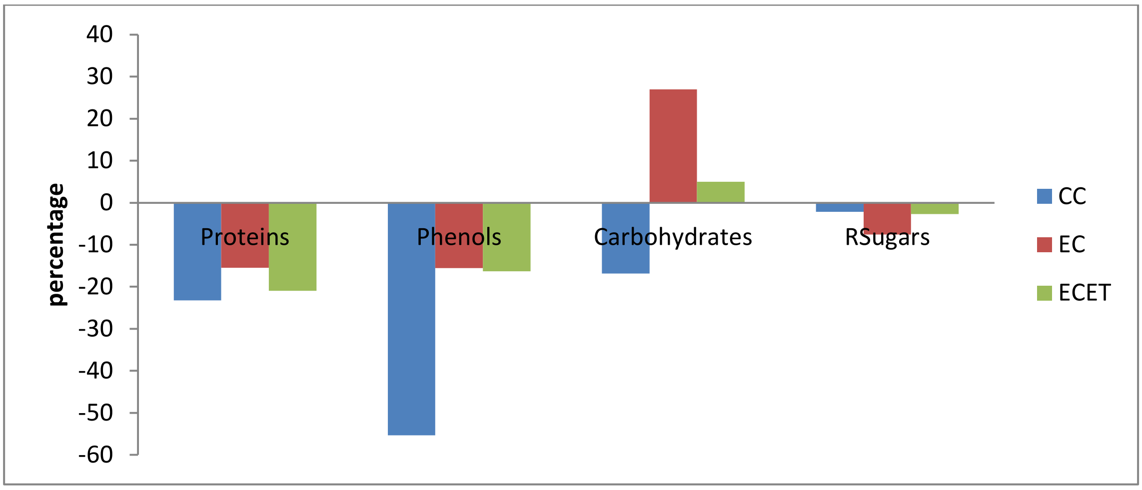

Figure10: Increase or decrease in proteins, phenol, carbohydrate and reducing sugar of *Casuarina equisetifolia* clones for different treatments (CC, EC, and ECET) as percentage over that of ambient condition.

Due to CO_2 fertilization the amount of carbohydrates produced in the elevated CO_2 plants were higher, when compared to control plants. Only EC plants shows highest carbohydrate production as it was hampered by the heat stress induced in the EC ET plants. Similarly, under the control plants, ambient shows relatively higher carbohydrate production than CC as the former was not subjected to any heat stress. Many studies shows that plants grown in elevated CO_2 usually had increased soluble sugar and starch content in leaves because of higher assimilation rates of carbohydrate consumption (Delucia *et al*., 1985). Such accumulation of total carbohydrates under elevated CO_2 levels was also reported by several authors with increases of 26to 50per cent (Griffin *et al*., 2000); Wurth *et al*. (1998); Wiemken and Ineichen, (2000).

Due to heat stress, the CC and EC ET produced high amounts of reducing sugars for combating the reactive oxygen species which arised due to the stress which caused the amount of reducing sugar to be high in these plants.For this very same reason the amount of reducing sugars were low in both Ambient and EC plants. But in contrast to results, Vu *et al*. (1989) recorded that the reducing sugars increased by 33per cent in soybean under 800 ppm of CO_2 enrichment.

Table 6: Total Chlorophyll in *Casuarina junghuhniana and Casuarina equisetifolia* for different treatments (Ambient, CC, EC, and ECET)

Species	treatment	total chlorophyll (mg/g)
CJ	Amb	16.68
	CC	16.89
	EC	16.40
	ECET	13.53
CE	Amb	15.95
	CC	16.39
	EC	15.84
	ECET	13.40

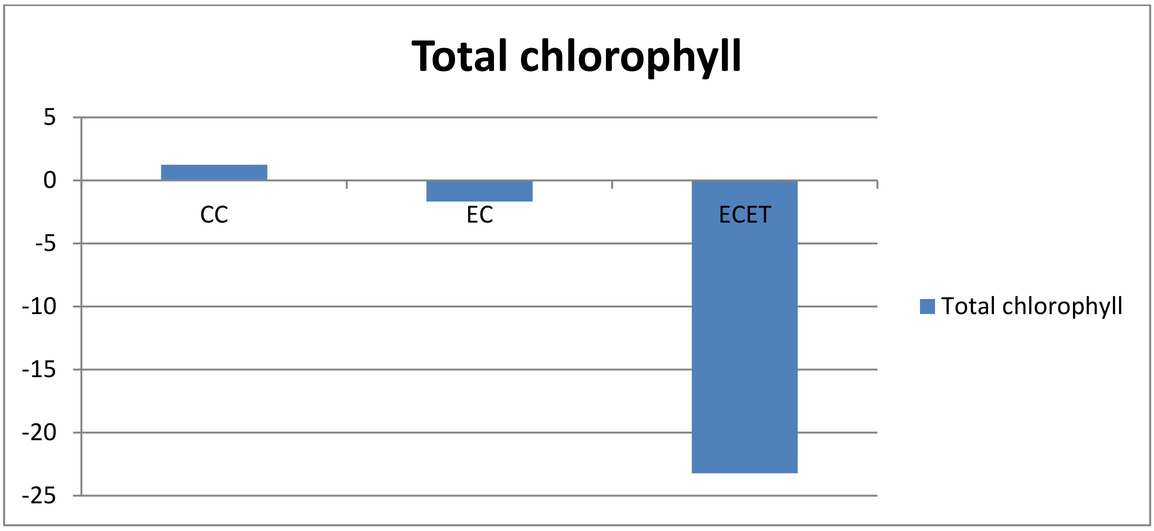

Figure 11: Increase or decrease in Total chlorophyll of *Casuarina junghuhniana* clones for different treatments (CC, EC, and ECET) as percentage over that of ambient condition.

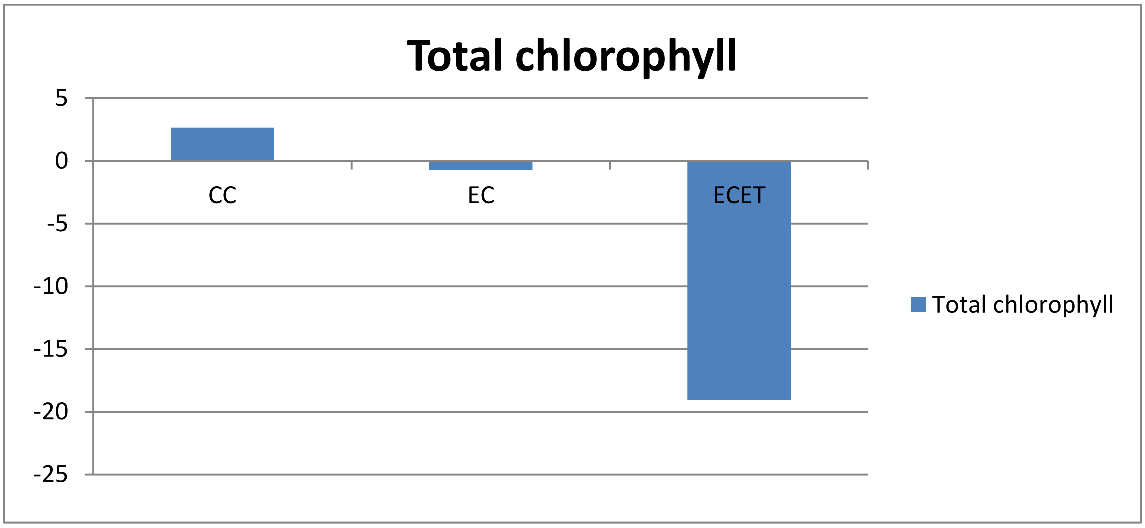

Figure 12: Increase or decrease in Total chlorophyll of *Casuarina equisetifolia* clones for different treatments (CC, EC, and ECET) as percentage over that of ambient condition.

Chlorophyll pigment density was significantly different in all the three clones of Casuarina under CO_2 exposure in automated open top chambers.

Table 7: ANOVA for various growth parameters of *Casuarina junghuhniana* and *Casuarina equisetifolia* clones

Parameters	Mean Sum of Squares								
	df	Shoot Length	Root Length	Needle Length	Needle diameter	Root Number	Collar Diameter	Leaf area	SVI
Days	1	7033.97***	13703.40***	421.40***	757.25***	757.25***	34.76**	3.3	659.30***
Treatment	3	75.01	5.15	33.31**	3.12	3.12	0.58***	3.0	2.43
Clones	11	492.80***	254.05***	152.04***	23.31***	23.31***	2.67***	12.7	19.19***
Days * Treatment	3	75.01	5.15	1.94	3.12	3.12	0.39*	1.5	7.92***
Days * Clones	11	685.76***	211.19***	35.50***	52.28***	52.28***	0.79***	2.6	13.30***
Treatment* Clones	33	32.63	16.04**	16.35***	5.58	5.58	1.04***	1.2	4.85***
Days* Treatment * Clones	33	32.63	16.04**	21.85***	5.58	5.58	0.37***	1.2	2.44**
Error	192	17.62	33.67	7.57	6.59	6.59	0.29	0.5	1.42

***P<0.001; ** P < 0.01; * P < 0.05

Table 8: ANOVA for various growth parameters of *Casuarina junghuhniana* and *Casuarina equisetifolia* clones

Parameters	Mean Sum of Squares								
	df	LFW	LFW Allocation	RFW	RFW Allocation	Fresh Weight RSR	SFW	SFW Allocation	TFW
Days	1	1312.04***	0.43***	779.37***	1.83***	186.05***	129.44***	0.50***	5750.92***
Treatment	3	2.66	0.01	5.51**	0.02	2.52**	0.01	0.00	11.86*
Clones	11	16.81***	0.13***	5.05***	0.04***	7.34***	2.17***	0.04***	49.42***
Days* Treatment	3	2.66	0.01	5.51**	0.02	2.52**	0.01	0.00	11.86*
Days* Clones	11	2.50*	0.10***	4.58***	0.02***	3.65***	0.62**	0.07***	15.98***
Treatment* Clones	33	2.29**	0.00	2.40***	0.01	1.56***	0.52**	0.00	10.82***
Days* Treatment* Clones	33	2.29**	0.00	2.40***	0.01	1.56***	0.52**	0.00	10.82***
Error	192	1.19	0.00	1.06	0.01	1.50	0.26	0.01	4.54

From the table it was noted that all parameters except leaf area were significant with respect to days of the treatment. CO_2 levels were significant at $p < .01$ level in needle diameter and $p<.001$ level in collar diameter. Clonal variations were significant at $p < .001$ level in all parameters except Leaf Area. The interaction effect of treatment and clones were significant at .01 level at root length and .001 significant in needle diameter, collar diameter and stem volume index.

This table indicates that CO_2 levels were significant at $p < .05$ level in fresh weight and $p<.001$ level in RFW and fresh weight RSR. Clonal variations were significant at $p < .001$ level for all parameters. The interaction effect of treatment and clones were significant at 0.01 levels at RFW and SFW and 0.001 significant RFW, RSR and TFW.

Table 9: ANOVA for various growth parameters of *Casuarina junghuhniana* and *Casuarina equisetifolia* clones

Parameters	Mean Sum of Squares								
	Df	LDW	LDW Allocation	RDW	RDW Allocation	Dry Weight RSR	SDW	SDW Allocation	TDW
Days	1	184.4***	0.77***	124.70***	2.14***	65.87**	30.01***	0.32***	913.75***
Treatment	3	0.5	0.01	0.44*	0.01	6.27	0.00	0.00	1.60
Clones	11	3.1***	0.03***	1.05***	0.02*	9.43	0.76***	0.03***	12.37***
Days* Treatment	3	0.5	0.01	0.44*	0.01	6.27	0.00	0.00	1.60
Days* Clones	11	1.0***	0.05***	0.61***	0.02***	10.36	0.17*	0.03***	3.78***
Treatment * Clones	33	0.5*	0.00	0.27*	0.00	7.97	0.13***	0.00	1.85**
Days* Treatment * Clones	33	0.5*	0.00	0.27*	0.00	7.97	0.13***	0.00	1.85**
Error	192	0.3	0.01	0.16	0.01	7.97	0.06	0.00	0.95

Table 10: ANOVA for various growth parameters of *Casuarina junghuhniana* and *Casuarina equisetifolia* clones

Parameters	Mean Sum of Squares								
	df	Chlorophyll A/B ratio	Carbohydrates	Chlorophyll A	Chlorophyll b	Phenols	Proteins	Reducing sugar	Total Chlorophyll
Days	1	5.1***	92234.1***	408.6***	1041.9***	1025.7***	862.9***	239158.2***	860.7***
Treatment	3	4.7***	12320.2***	16.0***	201.2***	79.8***	5861.5***	6787.0***	153.7***
Clones	11	0.3***	1484.8***	226.2***	132.2***	204.8***	39634.0***	10603.2***	115.7***
Days* Treatment	3	4.7***	3687.5***	1303.3***	695.2***	61.0***	6165.4***	10775.6***	602.7***
Days* Clones	11	0.3***	508.1***	39.1***	28.2***	20.4***	3456.0***	2632.5***	24.0***
Treatment* Clones	33	0.4***	334.1***	40.9***	9.8***	8.2***	751.4***	2063.4***	8.6***
Days* Treatment* Clones	33	0.3***	521.8***	51.0***	20.6***	6.9***	961.2***	918.3***	17.9
Error	192	0.0	38.7	0.1	0.0	0.6	126.0	273.2	0.0

4.2 INTRA - SPECIFIC VARIATIONS AFTER THREE MONTHS OF STUDY

The results obtained on variation in root length, shoot length, Collar diameter, primary roots (NR), RFW & RDW, SFW & SDW and LFW & LDW, in the 6 clones of *Casuarina equisetifolia and Casuarina junghuhniana* subjected to conditions of elevated temperature (CC), elevated CO_2 (EC) and the combined effects of elevated CO_2 + temperature (ECET) at the end of three months are presented below. Various derived parameters including total biomass, biomass partitioning in the root, shoot and leaves and RSR were also estimated during the study.

4.2.1 Intra-specific variations in morphological and biochemical parameters of *Casuarina junghuhniana*

4.2.1.1 Number of roots of *Casuarina junghuhniana* for different treatments

The responses of different clones of *Casuarina junghuhniana* show variations under different treatments. At the end of 3 months, CJ6 shows higher number of roots (8.5, 8.33, 8.7.33) and CJ8 show the lowest number of roots (5.67, 4.33, 2.83, and 4.17). Among the clones CJ 6 shows good response and among the treatment Ambient shows better growth.

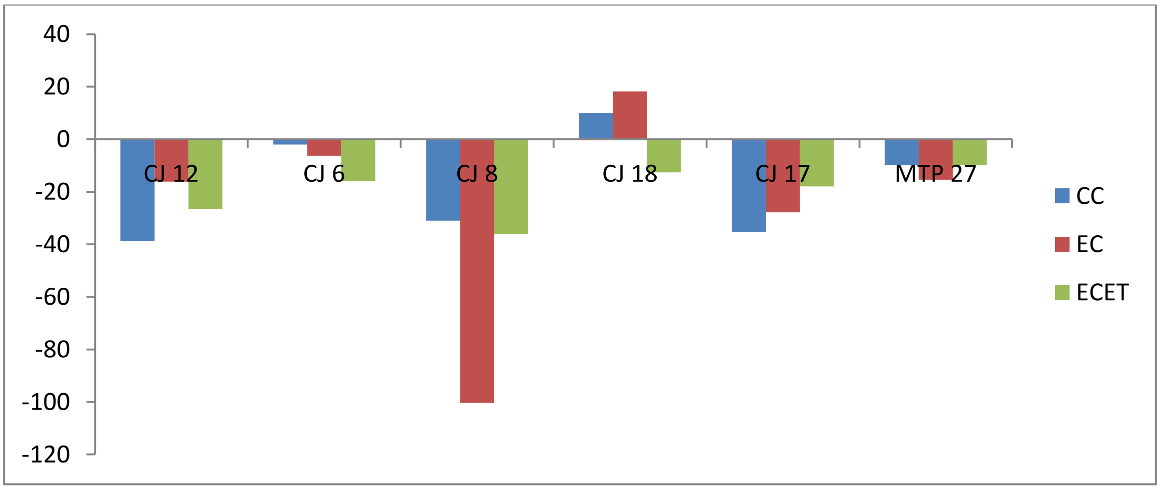

Figure 13: Increase or decrease in no. of roots of *Casuarina junghuhniana* for different treatments (CC, EC, and ECET) as percentage over that of ambient

Table 11: Differential growth response (no of roots, root length, shoot length) of clones of *Casuarina junghuhniana* to different treatments (Ambient, CC, EC and ECET)

Clones	Number of roots					Clones	Root Length (cm)					Clones	Shoot Length (cm)				
	Ambient	CC	EC	EC ET	Mean		Ambient	CC	EC	EC ET	Mean		Ambient	CC	EC	EC ET	Mean
CJ 12	7.17	5.17	6.17	5.67	6.05	CJ 12	20.7	18.05	20.28	19.83	19.72	CJ 12	23.00	20.60	21.95	26.93	23.12
CJ 6	8.50	8.33	8.00	7.33	8.04	CJ 6	17.58	17.92	18.92	17.92	18.09	CJ 6	18.83	18.10	24.27	21.38	20.65
CJ 8	5.67	4.33	2.83	4.17	4.25	CJ 8	19.33	17.50	16.72	23.75	19.33	CJ 8	26.28	27.6	33.45	29.27	29.15
CJ 18	6.00	6.67	7.33	5.33	6.33	CJ 18	21.42	21.17	23.07	24.12	22.45	CJ 18	25.88	25.63	30.9	29.02	27.86
CJ 17	7.67	5.67	6.00	6.5	6.46	CJ 17	18.42	20.75	19.75	16.42	18.84	CJ 17	25.38	26.18	26.92	27.33	26.45
MTP 27	7.5	6.83	6.50	6.83	6.92	MTP 27	27.58	28.25	28.75	29.75	28.58	MTP 27	35.57	32.00	32.8	32.17	33.14
Mean	7.09	6.17	6.14	5.97	6.34	Mean	20.84	20.61	21.25	21.97	21.16	Mean	25.82	25.02	28.38	27.68	26.73

Table 12: Differential growth response (collar diameter, needle length, needle diameter) of clones of *Casuarina junghuhniana* different treatments (Ambient, CC, EC and ECET)

Clones	Collar Diameter(mm)					Clones	Needle Length(cm)					Clones	Needle diameter(mm)				
	Amb	CC	EC	ECET	Mean		Amb	CC	EC	ECET	Mean		Amb	CC	EC	EC ET	Mean
CJ 12	4.15	2.85	3.26	3.39	3.41	CJ 12	17.95	14.37	16.08	16.35	16.19	CJ 12	0.07	0.06	0.08	0.07	0.07
CJ 6	4.20	3.73	3.73	3.47	3.78	CJ 6	19.46	19.81	20.09	17.14	19.13	CJ 6	0.08	0.08	0.07	0.08	0.08
CJ 8	4.48	3.74	3.56	3.64	3.86	CJ 8	16.75	17.25	18.76	17.03	17.45	CJ 8	0.07	0.07	0.07	0.08	0.07
CJ 18	3.60	3.20	4.02	3.01	3.46	CJ 18	13.36	18.10	16.38	11.25	14.77	CJ 18	0.08	0.05	0.06	0.06	0.06
CJ 17	4.02	2.90	3.70	3.04	3.42	CJ 17	12.09	15.49	13.95	13.35	13.72	CJ 17	0.07	0.06	0.06	0.06	0.06
MTP 27	3.92	4.63	4.66	4.15	4.34	MTP 27	23.05	18.88	23.38	21.31	21.66	MTP 27	0.07	0.07	0.07	0.07	0.07
Mean	4.06	3.51	3.82	3.45	3.71	Mean	17.11	17.32	18.11	16.07	17.15	Mean	0.07	0.07	0.07	0.07	0.07

4.2.1.2 Root Lengthof *Casuarina junghuhniana* for different treatments.

At the end of three months, ambient conditions shows a range of 17.58 (CJ6) to 27.58 (MTP 27), CC recorded higher values in MTP 27(28.25) and the lowest in CJ8 (17.50). Under EC condition, MTP 27 recorded the highest value (28.75) while the least value was recorded in CJ8 (16.72). Under ECET MTP 27 recorded the highest (29.75), while CJ17 (16.42) recorded the lowest value. Among the clones MTP 27 shows good response and among the treatment ECET shows better root length.

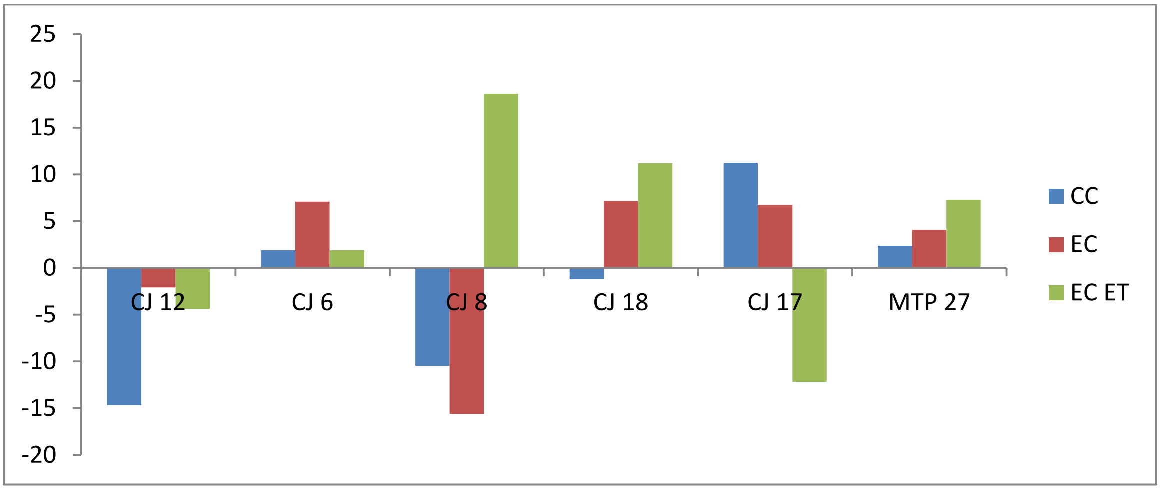

Figure 14: Increase or decrease in Root Length of *Casuarina junghuhniana* for different treatments (CC, EC, and ECET) as percentage over that of ambient condition.

4.2.1.3 Shoot lengthof *Casuarina junghuhniana* for different treatments

Shoot length varied significantly between treatments at 5 per cent level. At the end of three months, MTP 27 recorded the maximum shoot length (35.57) and the shortest clone observed was CJ6 (18.83) under ambient conditions. Under CC, CJ6 (18.10) were the lowest and MTP 27(32) was thehighest. CJ8 shows the highest shoot length (33.45) and CJ 12 shows the lowest shoot length (21.95) under EC condition. While under ECET, MTP 27recorded the highest and CJ6 recorded the lowest (32.17cm and 21.38cm) measurements respectively. Among the clones MTP 27 shows good response and

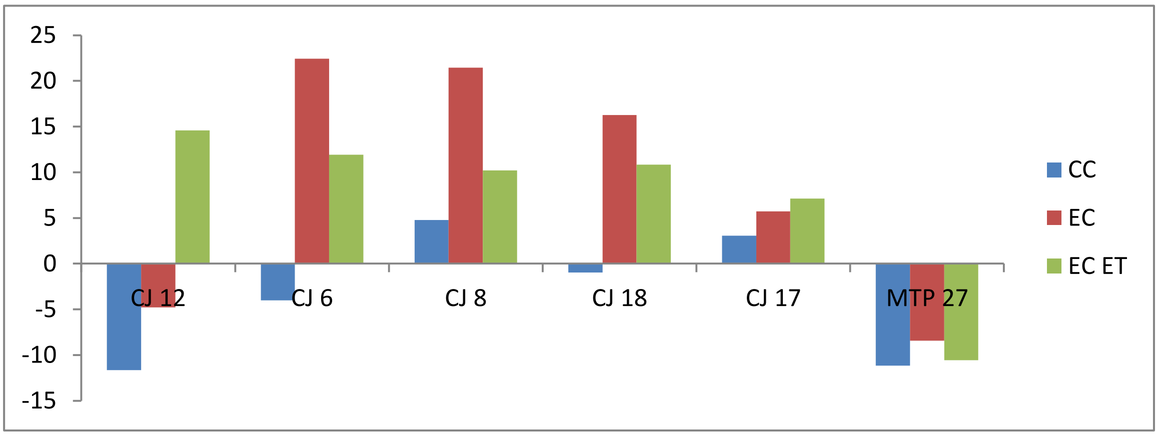

Figure 15: Increase or decrease in Shoot Length of *Casuarina junghuhniana*for different treatments (CC, EC, and ECET) as percentage over that of ambient condition.

4.2.1.4 Collar diameterof *Casuarina junghuhniana* for different treatments

At the end of 3 months, in ambient conditionsCJ8 recorded the maximum (4.48) while the least was recorded by CJ18 (3.60). MTP 27 ranked the highest (4.63) while CJ12 was again the lowest (2.85) under CC. However, under EC, MTP 27 ranked the highest (4.66) and CJ12 ranked the lowest (3.26). MTP 27 shows the highest Collar diameter (4.15) and CJ18 recorded the lowest (3.01) under EC ET condition. Among the clones MTP 27 shows good response and among the treatment ambient shows good response for collar diameter.

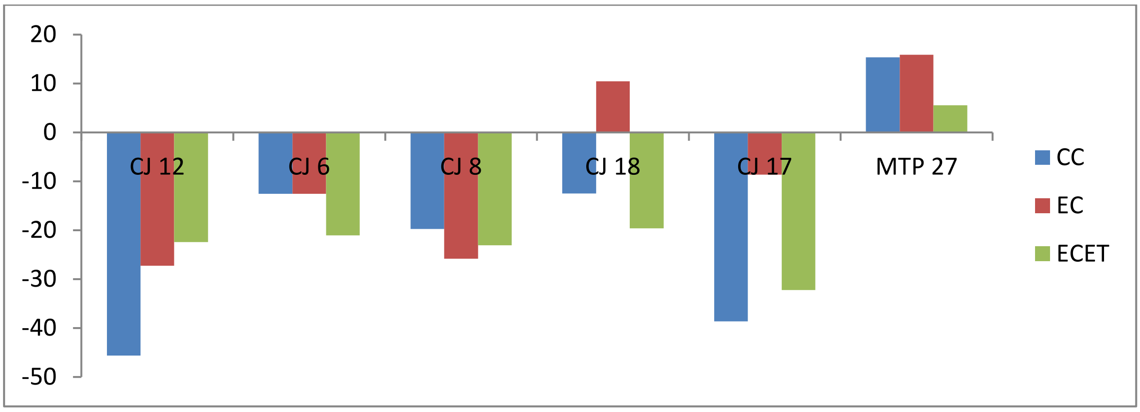

Figure 16: Increase or decrease in collar diameter of *Casuarina junghuhniana* for different treatments (CC, EC, and ECET) as percentage over that of ambient condition.

4.2.1.5 Needle Lengthof *Casuarina junghuhniana* for different treatments

At the end of 3 months, in ambient conditionsMTP 27 recorded the maximum (23.05) and CJ17 (12.09) recorded the minimum needle length. CJ6 ranked the highest (19.81) while CJ17was the lowest (15.49) under CC. However, under EC, MTP 27 ranked the highest (23.38) and CJ17 ranked the lowest (13.95). MTP 27shows the highest needle length (21.31) whereas CJ18 recorded the lowest needle length (11.25) under EC ET condition. Among the clones MTP 27 shows good response and among the treatment EC shows better results.

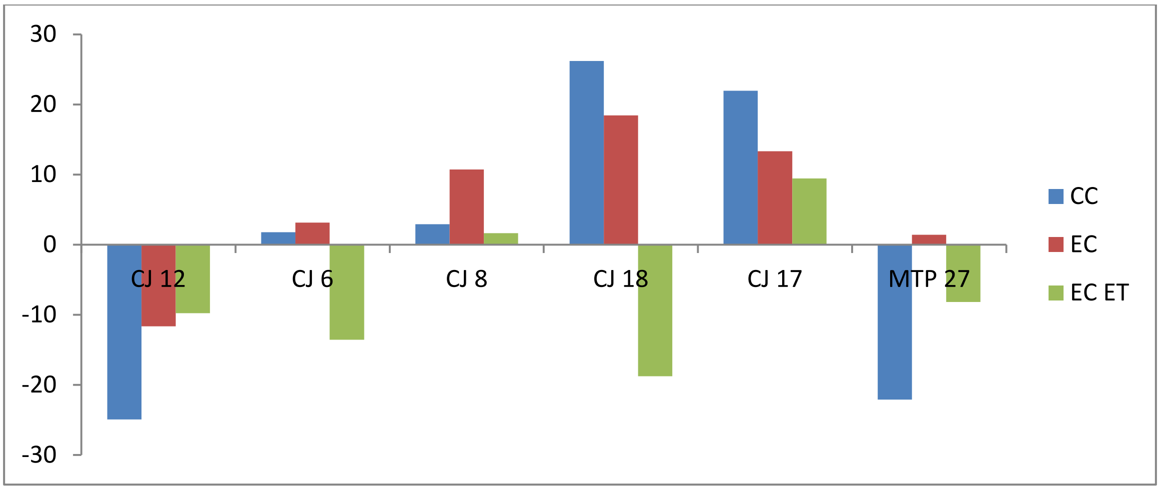

Figure 17: Increase or decrease in needle length of *Casuarina junghuhniana* for different treatments (CC, EC, and ECET) as percentage over that of ambient condition.

4.2.1.6 Needle diameterof *Casuarina junghuhniana* for different treatments

In ambient condition CJ18 andCJ6 recorded the maximum (0.08) while other clones recorded a lower value (0.06). CJ6 ranked the highest (0.08) while CJ6was again the lowest (0.05) under CC. However, under EC, CJ12ranked the highest (0.08) and CJ18 and CJ 17 rankedthe lowest (0.06). CJ8 andCJ6 recorded the highest needle diameter (0.08), while CJ18 and CJ 17 recorded the lowest needle diameter (0.06) under ECET condition. Among the clones CJ 6 shows good response and there is no significant difference between the treatments.

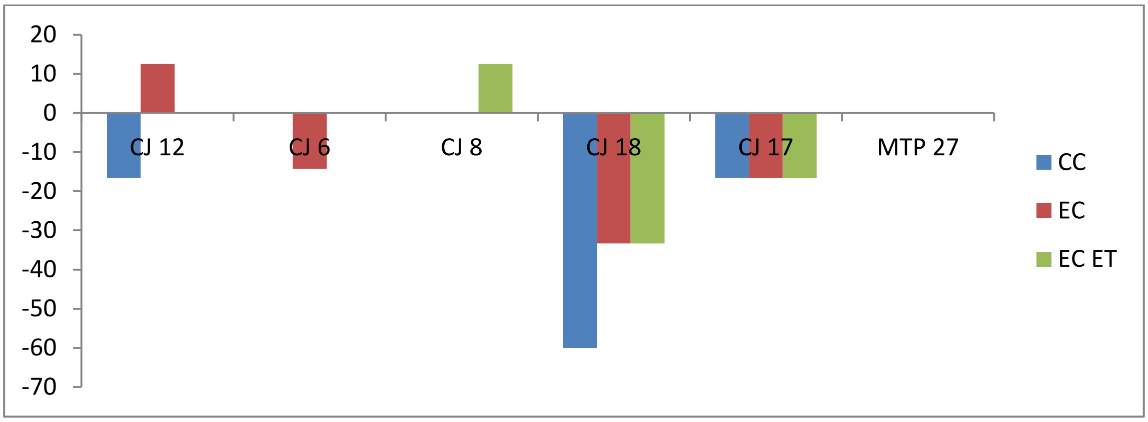

Figure 18: Increase or decrease in Needle Diameter of *Casuarina junghuhniana* for different treatments (CC, EC, and ECET) as percentage over that of ambient condition.

Table13: Differential growth response (leaf area and stem volume index) of clones of *Casuarina junghuhniana to* elevated CO_2

Clones	Leaf Area(mm^2)					Clones	Stem Volume Index				
	Ambient	CC	EC	ECET	Mean		Ambient	CC	EC	ECET	Mean
CJ 12	3.8	2.64	3.6	3.34	3.35	CJ 12	4.27	1.75	2.47	3.17	2.92
CJ 6	4.5	4.52	4.47	5.38	4.72	CJ 6	5.22	3.66	3.76	3.31	3.99
CJ 8	3.72	3.7	4.43	4.01	3.97	CJ 8	5.21	3.53	4.37	3.4	4.13
CJ 18	3.09	3.02	3.18	2.25	2.89	CJ 18	3.09	2.06	5.59	2.75	3.37
CJ 17	2.61	2.89	3.08	2.95	2.88	CJ 17	2.65	2.3	4.53	3.2	3.17
MTP 27	5.19	4.33	4.93	4.75	4.80	MTP 27	4.78	5.44	5.7	4.1	5.01
Mean	3.82	3.52	3.95	3.78	3.77	Mean	4.20	3.12	4.40	3.32	3.76

4.2.1.7 Leaf Areaof *Casuarina junghuhniana* for different treatments

MTP 27 recorded the maximum leaf area (5.19) while the least was recorded in CJ17 (2.61). Under ET CJ6 ranked highest (4.52) while CJ12 ranked the lowest (2.64). Under EC, MTP27recorded the highest (4.93) and CJ17 recorded the lowest (3.08). However, under ECET, CJ6 ranked the highest (5.38) and CJ18 ranked the lowest (2.25). Among the clones CJ 6 shows good response and among the treatment EC shows good in leaf area.

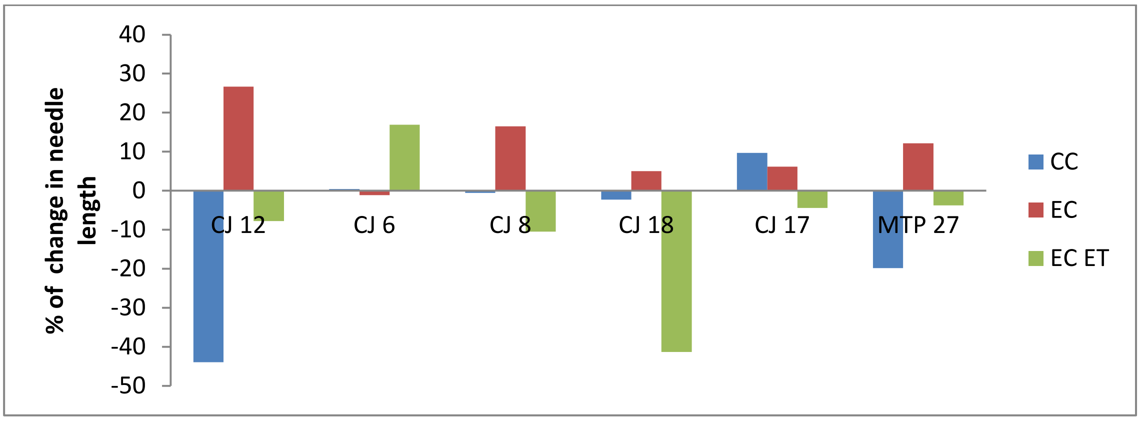

Figure 19: Increase or decrease in leaf area of *Casuarina junghuhniana* for different treatments (CC, EC, and ECET) as percentage over that of ambient condition.

4.2.1.8 Stem Volume Indexof *Casuarina junghuhniana* for different treatments

At the end of 3 months, in ambient conditions, CJ6recorded the maximum (5.22) while CJ18 (2.25) recorded the least stem volume index. MTP 27 ranked the highest (4.63) andCJ12 ranked the lowest (1.75) under ET. However under EC, MTP 27 ranked the highest (5.70) and CJ12recorded the lowest value (2.47). MTP 27 measured the highest SVI (4.10) and CJ18 measured the lowest (2.75) under EC ET condition. Among the clones MTP 27 shows good response and among the treatment EC shows good for stem volume index.

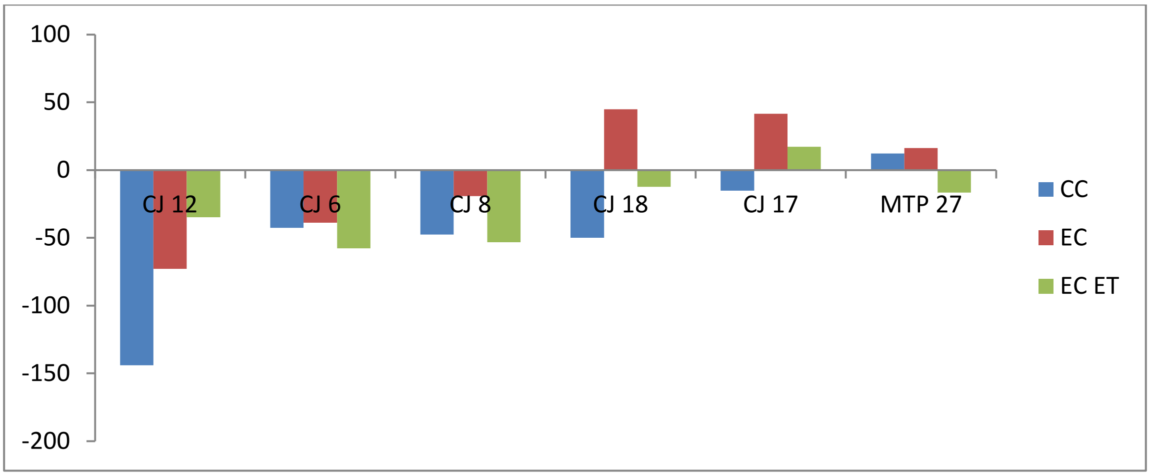

Figure 20: Increase or decrease in Stem Volume Index of *Casuarina junghuhniana* for different treatments (CC, EC, and ECET) as percentage over that of ambient condition.

4.2.1.9 Fresh weightof *Casuarina junghuhniana* for different treatments

Clones CJ12 and CJ6 had the lowest RFW (1.91 g) under ambient conditions, while MTP 27 recorded the highest (4.13 g). The highest RFW were recorded in MTP 27 (2.21g) under ET and, CJ18 under EC (3.92 g) and ECET (2.44 g). Lowest SDW was recorded in CJ12 (0.84 g) under ET conditions. The lowest under EC and ECET were recorded in CJ12 (0.77 g) and CJ6 (1.28 g) respectively. Among the clones CJ 18 shows good response and among the treatment Ambient shows good response for fresh weight.

Under ambient conditions, MTP 27recorded the highest SFW value (2.24g) while the lowest was recorded by CJ12 (2.24g). Under ET, the highest value of SFW was seen inCJ17 (1.76g) while it was the lowest in CJ6 (0.55g). Under EC, highest SFW was recorded in MTP 27 (1.87g) while the lowest was in CJ12 (0.52g). Among the clones MTP 27 shows good response and among the treatment Ambient shows good response for fresh weight.

Under ambient conditions, highest LFW was recorded in MTP 27 (25.11g) while the lowest was in CJ18 (2.84g). Under ET, MTP 27 shows the highest value (4.55g) and CJ12 recorded the lowest value (1.96g). Under EC, the highest LFW was recorded in CJ18 (5.73 g) while the lowest was recorded in CJ12 (1.94g). Under ECET, MTP 27 recorded the highest LFW (5.59g) whileCJ6 recorded the lowest (2.54g). Among the clones MTP 27 shows good response and among the treatment ambient shows good response for fresh weight.

4.2.1.10. Dry weightof *Casuarina junghuhniana* for different treatments

Clone CJ12 and CJ6 had the lowest RDW (0.84g) under ambient conditions, while MTP 27 recorded the highest value (1.50g). The highest RDW was recorded in MTP 27 (1.01g) under ET and, CJ18 under EC (1.28g) and ECET (1.16g). Lowest SDW was recorded in CJ12 (0.32g) under ET conditions while the lowest under EC and ECET was recorded in CJ12 (0.40g) and CJ6 (0.40g) respectively. Among the clones MTP 27 shows good response and among the treatment ambient shows good response for RDW.

Under ambient conditions, MTP 27 recorded the highest value (1.15 g) while the lowest was shown by CJ12 (0.47g). Under ET, the highest value of SDW was seen inCJ17 (0.96g) while it was the lowest in CJ6 (0.35 g). Under EC, the highest SDW was recorded in MTP 27 (1.08g) while the lowest was recorded in CJ12 (0.24 g). Among the clones MTP 27 shows good response and among the treatment ambient shows good response for SDW.

Under ambient conditions, the highest LDW was recorded in MTP 27 (2.05 g) while the lowest was seen in CJ18 (1.08 g). Under ET, MTP 27 shows the highest value (1.81 g) and CJ12 showsthe lowest (0.65g). Under EC, the highest LDW was recorded in CJ18 (1.96g) while the lowest was recorded in CJ12 (0.65g). Under ECET, MTP 27 recorded the highest LFW (1.99g) and CJ6 recorded the lowest (1.05g). Among the clones, MTP 27 shows good response and among the treatment Ambient shows good response for LDW.

Table 14: Differential growth response (fresh weight of root, shoot and leaf) of clones of *Casuarina junghuhniana to* elevated CO_2

Clones	FWR					Clones	FWS					Clones	FWL				
	Amb	CC	EC	ECET	Mean		Amb	CC	EC	EC ET	Mean		Amb	CC	EC	EC ET	Mean
CJ 12	1.91	0.84	0.77	1.52	1.26	CJ 12	1.14	0.55	0.52	1.10	0.83	CJ 12	3.1	1.96	1.94	3.53	2.63
CJ 6	2.14	1.76	1.82	1.58	1.83	CJ 6	1.12	0.9	0.97	0.85	0.96	CJ 6	3.55	3.04	3.38	2.54	3.13
CJ 8	4.13	2.08	1.62	1.28	2.28	CJ 8	1.41	1.15	1.02	1.20	1.20	CJ 8	4.87	3.82	4.3	3.61	4.15
CJ 18	2.35	1.54	3.92	1.76	2.39	CJ 18	0.96	0.98	1.61	1.03	1.15	CJ 18	2.84	3.00	4.4	4.16	3.60
CJ 17	2.69	1.62	2.06	1.31	1.92	CJ 17	1.19	1.76	1.07	0.89	1.23	CJ 17	4.29	2.63	3.45	2.95	3.33
MTP 27	2.04	2.21	2.11	2.44	2.20	MTP 27	2.24	1.51	1.87	1.64	1.82	MTP 27	5.11	4.55	5.73	5.59	5.25
Mean	2.54	1.68	2.05	1.65	1.98	Mean	1.34	1.14	1.18	1.12	1.20	Mean	3.96	3.17	3.87	3.73	3.68

Table 15:Differential growth response (dry weight of root, shoot, and leaf) of clones of *Casuarina junghuhniana* to elevated CO_2

Clones	DWR					Clones	DWS					Clones	DWL				
	Amb	CC	EC	ECET	Mean		Amb	CC	EC	EC ET	Mean		Amb	CC	EC	EC ET	Mean
CJ 12	0.84	0.32	0.40	0.70	0.57	CJ 12	0.47	0.41	0.24	0.53	0.41	CJ 12	1.20	0.65	0.65	1.32	0.96
CJ 6	0.84	0.89	0.82	0.50	0.76	CJ 6	0.62	0.35	0.51	0.38	0.47	CJ 6	1.36	1.16	1.28	1.05	1.21
CJ 8	1.41	0.86	0.75	0.55	0.89	CJ 8	0.67	0.49	0.52	0.62	0.58	CJ 8	2.02	1.56	1.52	1.37	1.62
CJ 18	0.93	0.54	1.28	1.16	0.98	CJ 18	0.52	0.55	0.86	0.56	0.62	CJ 18	1.08	1.13	1.96	1.74	1.48
CJ 17	1.07	0.84	0.79	0.76	0.87	CJ 17	0.59	0.96	0.54	0.55	0.66	CJ 17	1.83	1.16	1.29	1.32	1.40
MTP 27	1.50	1.01	1.23	1.05	1.20	MTP 27	1.15	0.79	1.08	0.87	0.97	MTP 27	2.05	1.81	2.46	1.99	2.08
Mean	1.10	0.74	0.88	0.79	0.88	Mean	0.67	0.59	0.63	0.59	0.62	Mean	1.59	1.25	1.53	1.47	1.46

Table 16: Differential growth response (total fresh weight, total dry weight, MC) of clones of *Casuarina junghuhniana* to elevated CO_2

Clones	FWT					Clones	DWT					Clones	MC				
	Amb	CC	EC	ECET	Mean		Amb	CC	EC	ECET	Mean		Amb	CC	EC	EC ET	Mean
CJ 12	6.15	3.35	3.22	6.16	4.72	CJ 12	2.51	1.39	1.29	2.54	1.93	CJ 12	59.19	58.51	59.94	58.77	59.10
CJ 6	6.58	5.49	5.94	4.75	5.69	CJ 6	2.82	2.40	2.61	1.93	2.44	CJ 6	57.14	56.28	56.06	59.37	57.21
CJ 8	10.41	7.04	6.94	6.10	7.62	CJ 8	4.10	2.91	2.78	2.54	3.08	CJ 8	60.61	58.66	59.94	58.36	59.39
CJ 18	6.14	5.53	9.93	6.95	7.14	CJ 18	2.54	2.22	4.10	3.46	3.08	CJ 18	58.63	59.86	58.71	50.22	56.86
CJ 17	8.16	6.01	6.57	5.15	6.47	CJ 17	3.48	2.95	2.62	2.62	2.92	CJ 17	57.35	50.92	60.12	49.13	54.38
MTP 27	9.39	8.26	9.71	9.67	9.26	MTP 27	4.69	3.61	4.76	3.90	4.24	MTP 27	50.05	56.30	50.98	59.67	54.25
Mean	7.81	5.95	7.05	6.46	6.82	Mean	3.36	2.58	3.03	2.83	2.95	Mean	57.16	56.76	57.63	55.92	56.87

4.2.1.11. Total fresh weight of *Casuarina junghuhniana* for different treatments.

Clone CJ18 shows the maximum fresh weight (10.41 g) in ambient conditions. CJ18 shows a higher value (9.93 g) in EC while MTP 27 recorded the highest (9.67 g) in EC ET. The lowest fresh weight values were recorded in CJ12 clone (3.22 g) in EC, followed by ambient (6.14 g) and ET (3.35 g). Among the clones MTP 27 shows good response and among the treatment Ambient shows good response for total fresh weight.

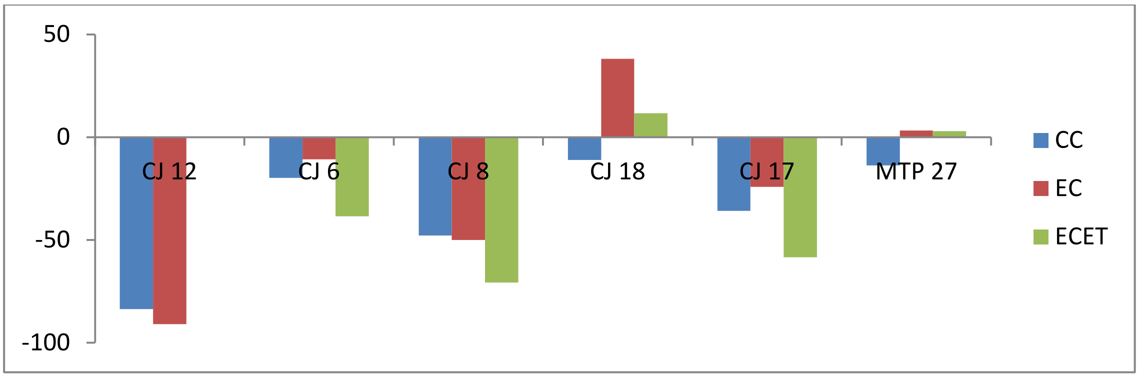

Figure 21: Increase or decrease in Fresh Weight of *Casuarina junghuhniana* for different treatments (CC, EC, and ECET) as percentage over that of ambient condition.

4.2.1.12 Total Dry weightof *Casuarina junghuhniana* for different treatments

Among the clones MTP 27 shows good response and among the treatment Ambient shows good response

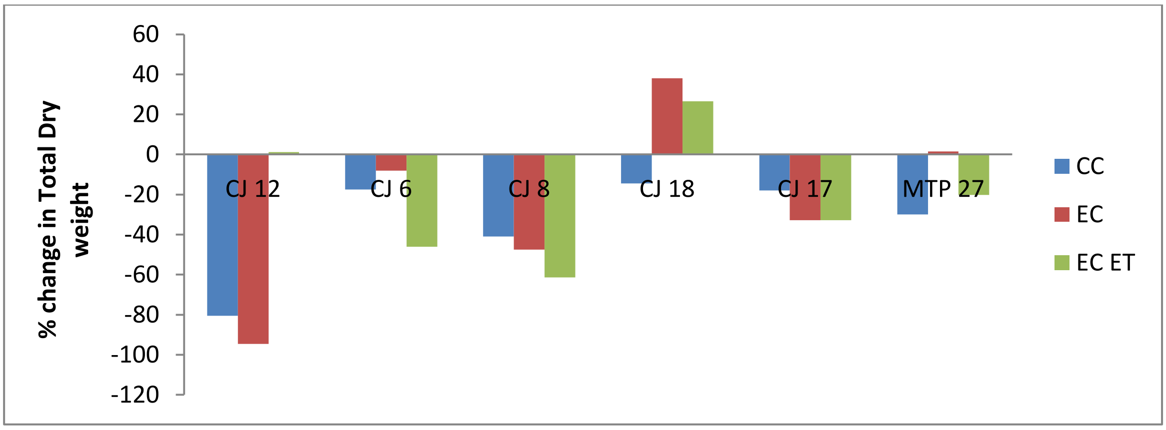

Figure 22: Increase or decrease in Total Dry Weight of *Casuarina junghuhniana* for different treatments (CC, EC, and ECET) as percentage over that of ambient condition.

Table 17: Differential growth response (protein, phenol) of clones of *Casuarina junghuhniana* to elevated CO_2

Clones	Proteins(mg/g)					Clones	Phenols(mg/g)				
	Amb	CC	EC	ECET	Mean		Amb	CC	EC	ECET	Mean
CJ 12	108.43	72.80	113.85	88.87	95.99	CJ 12	7.88	4.54	7.71	5.14	6.32
CJ 6	64.09	47.27	55.83	47.54	53.68	CJ 6	5.19	2.77	4.18	2.79	3.73
CJ 8	56.41	47.88	44.93	40.70	47.48	CJ 8	4.68	2.19	2.27	1.94	2.77
CJ 18	70.96	59.77	80.60	43.99	63.83	CJ 18	4.97	3.61	2.87	1.92	3.34
CJ 17	74.04	63.04	75.24	69.42	70.44	CJ 17	6.73	4.44	3.73	8.65	5.89
MTP 27	133.22	116.15	123.3	97.89	117.64	MTP 27	10.88	9.01	6.54	5.77	8.05
Mean	84.53	67.82	82.29	64.74	74.84	Mean	6.72	4.43	4.55	4.37	5.02

4.2.1.13 Proteinsof *Casuarina junghuhniana* for different treatments

At the end of 3 months in ambient conditions,MTP 27recorded the maximum protein content (133.22), while the least was recorded in CJ6 (64.09). MTP 27 ranked the highest (116.15) while CJ6was again the lowest (47.27) under ET. However under EC, MTP 27 ranked the highest (123.30) and CJ8 ranked the lowest (44.93). MTP 27 shows the highest protein content (97.89) and CJ8 shows the lowest protein content (40.70) under EC ET condition. MTP 27 shows good response and among the treatment ambient shows good response for proteins.

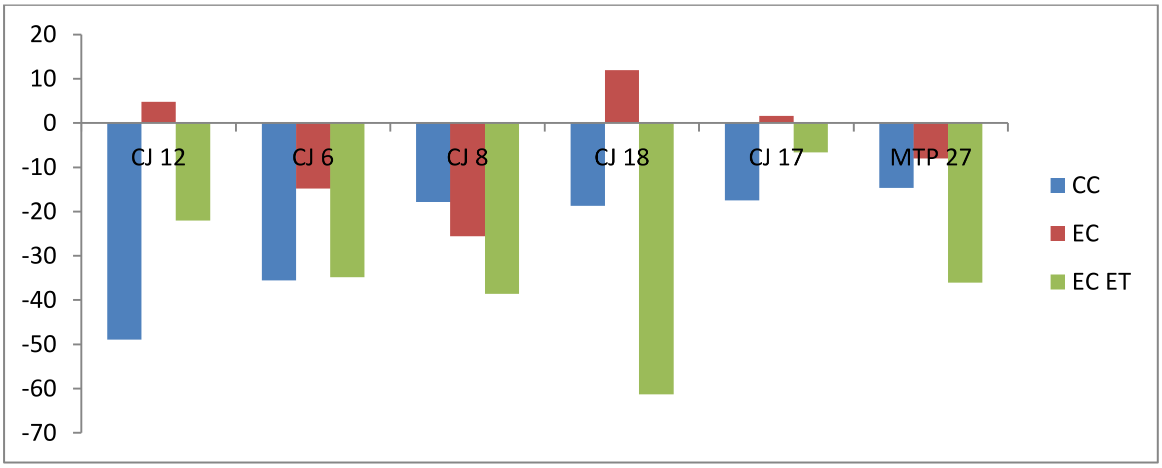

Figure 23: Increase or decrease in Protein of *Casuarina junghuhniana* for different treatments (CC, EC and ECET) as percentage over that of ambient condition.

4.2.1.14 Phenolsof *Casuarina junghuhniana* for different treatments.

At the end of 3 months in ambient conditions,MTP 27 recorded the maximum phenol content (10.88) while the least phenol content was recorded in CJ8 (4.68). MTP 27 ranked the highest (9.01) while CJ6 ranked the lowest (2.77) in phenol content under ET. However under EC, CJ12 recorded the highest (7.71) and CJ8 recorded the lowest values (2.27). CJ17 shows the highest phenol content (8.65) and CJ18 shows the lowest value (1.92) under EC ET condition. Among the clones MTP 27 shows good response and among the treatment Ambient shows good response for phenols.

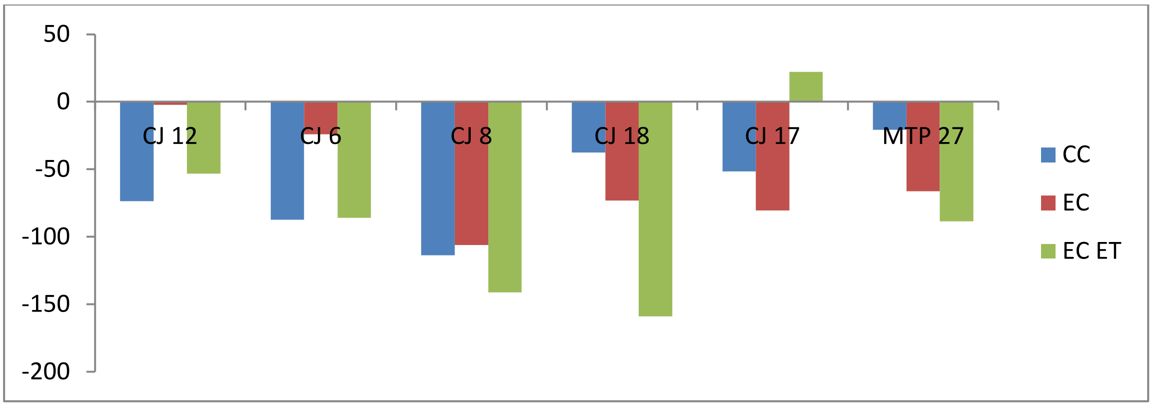

Figure 24: Increase or decrease in Phenol of *Casuarina junghuhniana* for different treatments (CC, EC, and ECET) as percentage over that of ambient condition.

Table 18: Differential growth response (carbohydrate and reducing sugar) of clones of *Casuarina junghuhniana* to elevated CO_2

Clones	Carbohydrates					Clones	Reducing Sugars				
	Amb	CC	EC	ECET	Mean		Amb	CC	EC	ECET	Mean
CJ 12	56.87	51.5	83.09	76.6	67.015	CJ 12	398.87	394.03	390.94	430.15	403.50
CJ 6	61.95	56.84	83.04	73.84	68.9175	CJ 6	344.12	396.87	379.02	387.66	376.92
CJ 8	57.65	58.69	88.19	72.89	69.355	CJ 8	315.37	384.16	368.64	370.8	359.74
CJ 18	68.59	61.33	106.21	72.04	77.0425	CJ 18	395.62	406.2	384.21	399.08	396.28
CJ 17	70.15	65.97	94.95	78.65	77.43	CJ 17	435.67	438.43	408.7	439.73	430.63
MTP 27	60.4	73.57	93.33	79.32	76.655	MTP 27	426.5	416.09	387.62	430.88	415.27
Mean	62.60	61.32	91.47	75.56	72.74	Mean	386.03	405.96	386.52	409.72	397.06

4.2.1.15 Carbohydratesof *Casuarina junghuhniana* for different treatments

At the end of three months carbohydrate amount is higher in elevated CO_2 condition in all the clones. In ambient condition, CJ17 recorded the maximum value (70.15) and CJ8 recorded the minimum value of (57.65). Whereas under CC, MTP 27rankedhighest (73.57) and CJ8 ranked the lowest (384.16).Under EC, CJ17 recorded the highest (408.70) and CJ8 recorded the lowest values (368.64). Under EC ET conditions CJ17 shows the highest value of reducing sugar (439.73) whereas CJ8 shows the lowest value (370.80). Among the clones CJ 18 shows good response and among the treatment EC shows good response for carbohydrates.

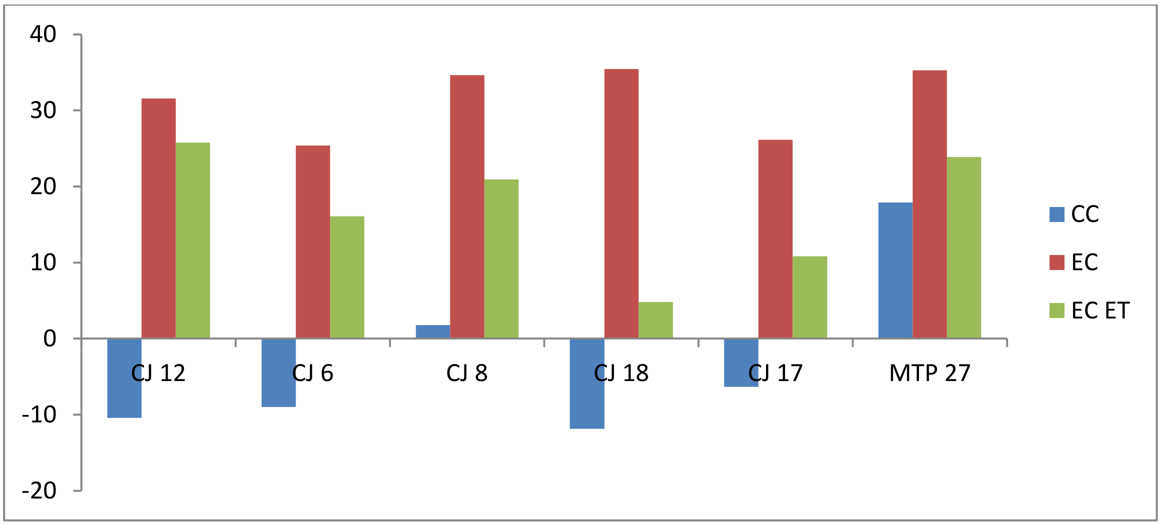

Figure 25: Increase or decrease in Carbohydrate of *Casuarina junghuhniana* for different treatments (CC, EC, and ECET) as percentage over that of ambient condition.

4.2.1.16 Reducing Sugarsof *Casuarina junghuhniana* for different treatments

At the end of 3 months in ambient conditions, CJ17recorded the maximum value (435.67) andCJ8recorded the minimum value of reducing sugars (315.37). Whereas under CC, CJ17ranked highest (438.43) and CJ8 ranked the lowest (384.16).Under EC, CJ17 recorded the highest (408.70) and CJ8 recorded the lowest values (368.64). Under EC ET conditions CJ17 shows the highest value of reducing sugar (439.73) whereas CJ8 shows the lowest value (370.80). Among the clones CJ 17 shows good response and among the treatment ambient shows good response for reducing sugars.

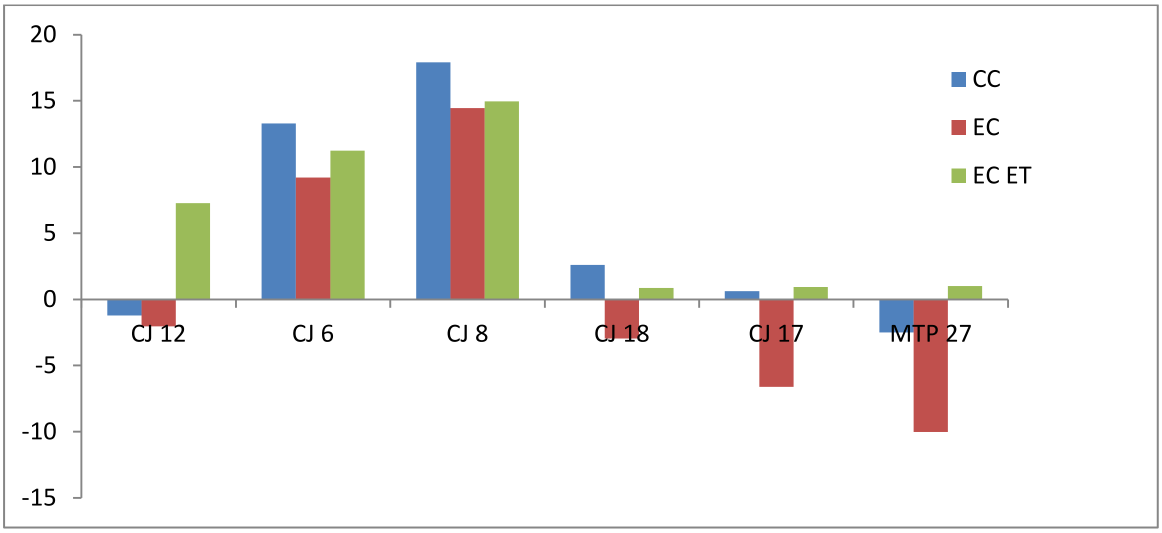

Figure 26: Increase or decrease in reducing sugars of *Casuarina junghuhniana* for different treatments (CC, EC, and ECET) as percentage over that of ambient condition.

4.2.1.17 Total Chlorophyllof *Casuarina junghuhniana* for different treatments

At the end of 3 months in ambient conditions,TCR 06-01-01 recorded the maximum value for total chlorophyll (18.31) while the least value was recorded in CE 02-01 (14.11). TCR 06-01-01 ranked the highest (21.03) while CE 02-01 was again ranked the lowest in total chlorophyll values (11.03) under ET. However under EC, TCR 06-01-01 ranked the highest (21.45) and CE 02-01 ranked the lowest (9.65) in terms of total chlorophyll. TCR 12-02-03 shows the highest total chlorophyll content (16.82) and CE 02-01 shows the lowest value of total chlorophyll (10.67) under EC ET conditions. Among the clones CJ 17 shows good response and among the treatment CC shows good response for total chlorophyll.

Table 19: Differential growth response (total chlorophyll) of clones of *Casuarina Junghuhniana* to elevated CO_2

Clones	Total chlorophyll				
	Amb	CC	EC	ECET	Mean
CJ 12	14.83	15.38	14.92	10.15	13.82
CJ 6	16.90	17.46	15.89	13.62	15.97
CJ 8	16.40	15.55	14.41	12.78	14.79
CJ 18	16.80	19.24	18.71	14.90	17.41
CJ 17	20.03	19.11	18.74	17.03	18.73
MTP 27	15.09	14.57	15.72	12.71	14.53
Mean	16.68	16.885	16.40	13.53	15.87

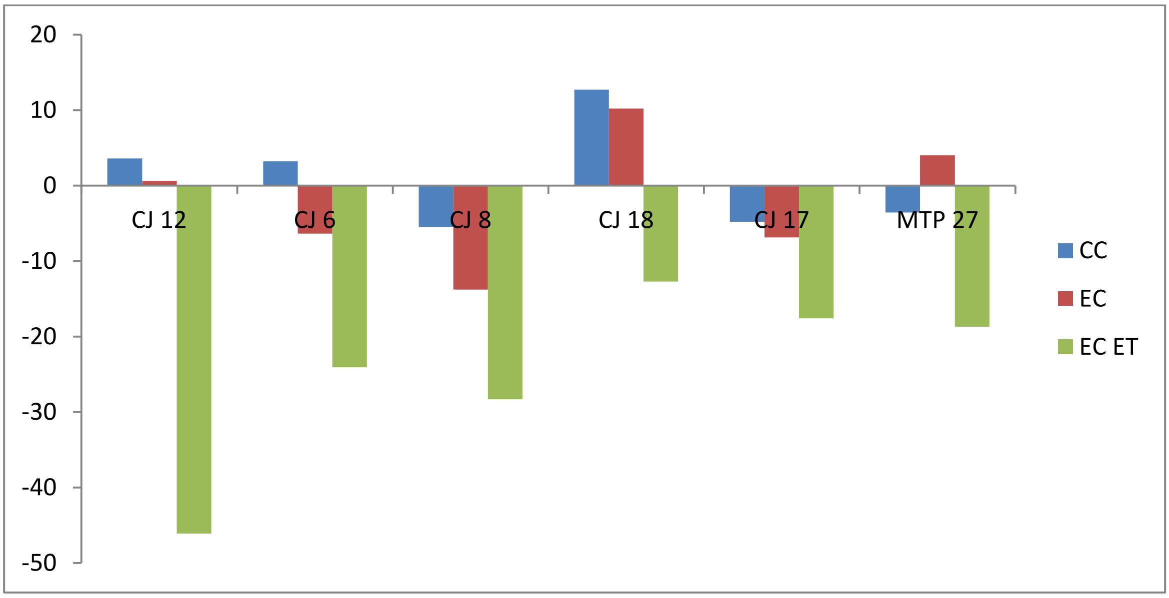

Figure 27: Increase or decrease in total chlorophyll of *Casuarina junghuhniana* for different treatments (CC, EC, and ECET) as percentage over that of ambient condition.

4.2.2 Intra-specific variations in morphological and biochemical parameters of *Casuarina equisetifolia*

Table 20: Differential growth response (no of roots, root length, shoot length) of clones of *Casuarina equisetifolia* to elevated CO_2

	Number of roots					Clones	Root Length					Clones	Shoot Length				
	Amb	CC	EC	ECET	Mean		Amb	CC	EC	EC ET	avg		Amb	CC	EC	EC ET	Mean
TCR 12-02-03	5.67	8.33	6.83	6.50	6.83	TCR 12-02-03	17.08	16.58	17.58	17.58	17.21	TCR 12-02-03	18.42	18.70	17.77	22.55	19.36
CE 02-04-03	6.67	5.5	8.17	7.33	6.92	CE 02-04-03	19.17	19.50	18.83	20.00	19.38	CE 02-04-03	19.47	23.70	19.50	22.55	21.31
CE-02-02-04	6.33	7.00	6.33	6.50	6.54	CE 02-02-04	17.17	17.67	13.58	18.33	16.69	CE-02-02-04	20.17	26.72	28.92	20.55	24.09
TCR 02-01-01	6.83	7.00	9.83	8.00	7.92	TCR 02-01-01	17	19.00	17.17	16.50	17.42	TCR 02-01-01	23.3	27.78	24.77	27.10	25.74
TCR 06-01-01	6.33	6.17	8.33	8.17	7.25	TCR 06-01-01	24.25	21.08	22.58	20.75	22.17	TCR 06-01-01	21.75	24.93	23.57	20.50	22.69
CE 02-01	7.50	6.33	6.83	8.50	7.29	CE 02-01	19.75	17.42	21.50	17.75	19.11	CE 02-01	16.28	16.25	17.62	17.58	16.93
Mean	6.56	6.72	7.72	7.50	7.12	Mean	19.07	18.54	18.54	18.49	18.66	Mean	19.90	23.01	22.03	21.81	21.69

4.2.2.1 Number of rootsof*Casuarina equisetifolia* for different treatments

The response of different clones of *Casuarina equisetifolia* shows variation under different treatments. At the end of 3 months, CE 02-01, TCR 12-02-03, TCR 02-01-01, CE 02-01 under ambient conditions, ET, EC and EC ET shows higher number of roots 7.50, 8.33, 9.83 and 8.50 respectively. At the same time, TCR 12-02-03, CE 02-04-03, CE 02-02-04 under ambient conditions, ET, EC and EC ET shows lower number of roots 5.67, 5.50, 6.33, and 6.50 respectively. Among the clones TCR 02.01.01shows good response and among the treatment EC shows good response in number of roots.

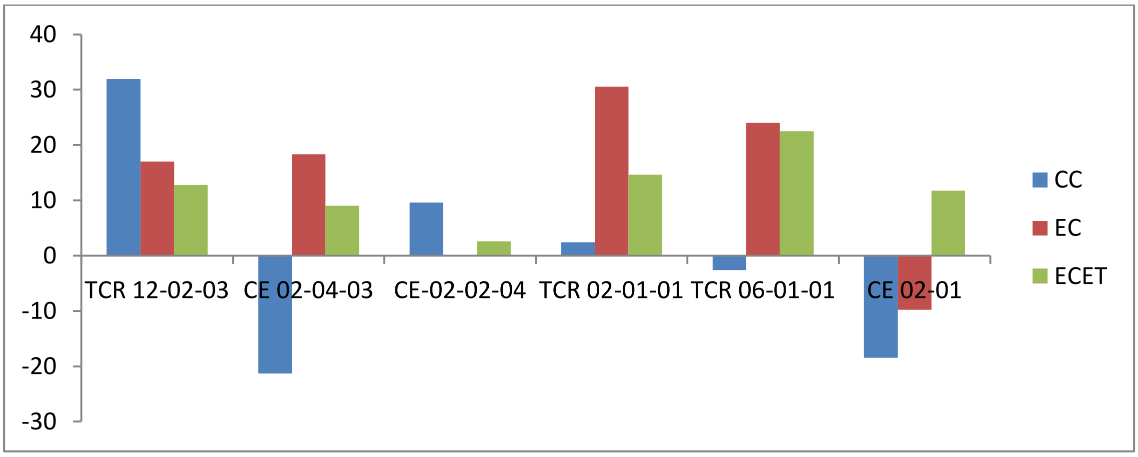

Figure 28: Increase or decrease in Root number of *Casuarina equisetifolia* for different treatments (CC, EC and ECET) as percentage over that of ambient condition.

4.2.2.2 Root lengthof*Casuarina equisetifolia* for different treatments

At the end of three months under ambient conditions, root lengthshows a wide range of variation from17.00 (TCR 02-01-01) to 24.25 (TCR 06-01-01). ET recorded higher value in TCR 06-01-01 (21.08) and the lowest in TCR 12-02-03 (16.58). Under EC condition TCR 06-01-01 recorded the highest (22.58) andCE 02-02-04 (13.58) recorded the lowest root length. Under EC ET, TCR 06-01-01 recorded the highest (20.75), while TCR 02-01-01 (16.50) recorded the lowest root length.TCR 06-01-01 shows good response and among the treatment ambient shows good response for root length.

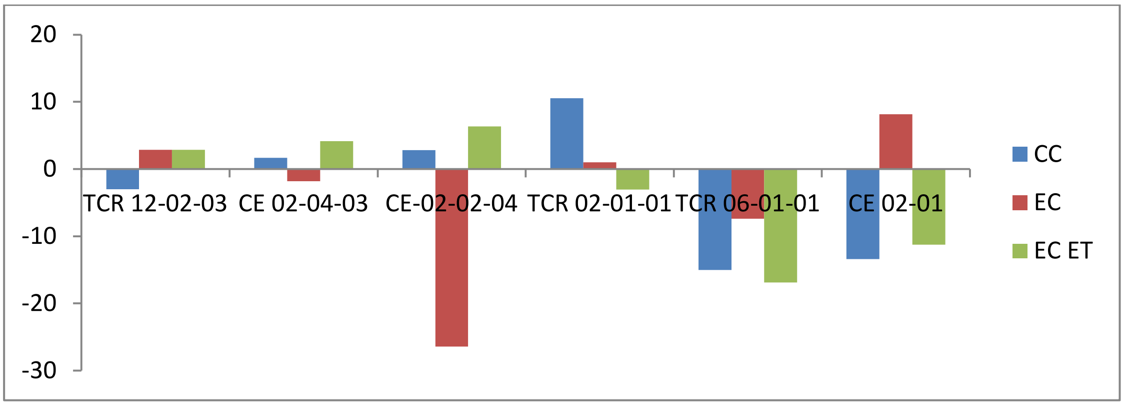

Figure 29: Increase or decrease in root length of *Casuarina equisetifolia* for different treatments (CC, EC and ECET) as percentage over that of ambient condition.

4.2.2.3 Shoot lengthof*Casuarina equisetifolia* for different treatments

Shoot length varied significantly among treatments at 5 per cent level. At the end of three months, TCR 02-01-01 recorded the maximum shoot length under ambient condition (23.30) and the lowest by CE-21(16.28) , CE 02-02-04 (28.92) under EC and the shortest clone observed was CE 02-01 (16.25) under ET., ET and ECET recorded higher values of shoot length 23.30, 27.28, 27.10 respectively. CE 02-01 recorded lower shoot length under all treatments (16.28, 16.25, 17.62, and 17.58) TCR 02.01.01shows good response and among the treatment CC shows good response

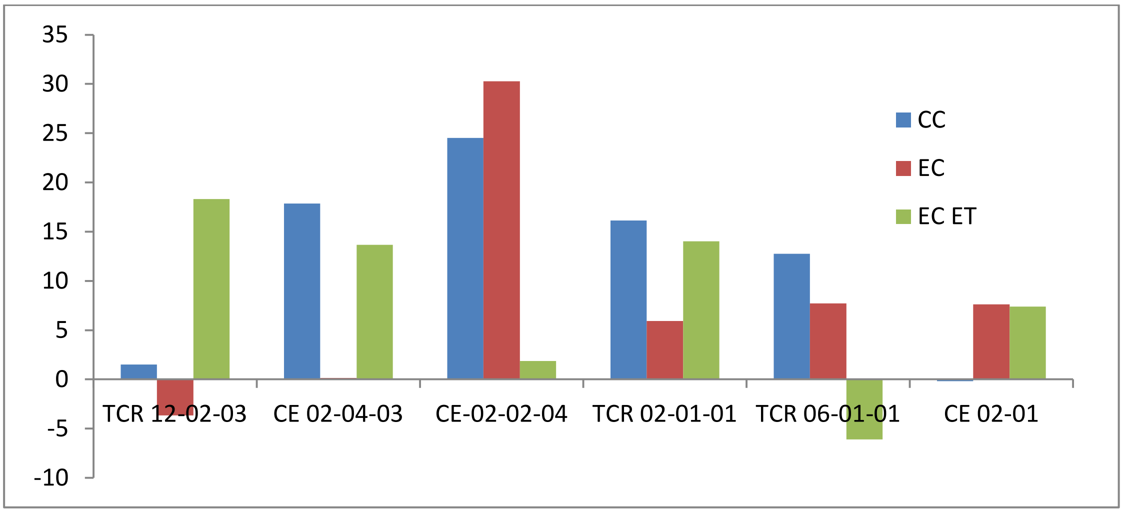

Figure 30: Increase or decrease in Shoot length of *Casuarina equisetifolia* for different treatments (CC, EC and ECET) as percentage over that of ambient condition.

Table 21: Differential growth response (collar diameter, needle length, needle diameter) of clones of *Casuarina equisetifolia* to elevated CO_2

	Amb	CC	EC	ECET	Mean		Amb	CC	EC	ECET	Mean		Amb	CC	EC	ECET	Mean
TCR 12-02-03	3.03	3.81	3.13	4.27	3.56	TCR 12-02-03	12.15	14.50	14.24	15.83	14.18	TCR 12-02-03	0.07	0.07	0.07	0.08	0.07
CE 02-04-03	3.53	3.77	3.30	2.86	3.37	CE 02-04-03	13.41	12.81	13.15	15.94	13.83	CE 02-04-03	0.06	0.07	0.07	0.07	0.07
CE 02-02-04	3.23	3.47	2.89	3.43	3.26	CE -02-02-04	14.88	15.27	15.40	14.51	15.02	CE 02-02-04	0.06	0.07	0.06	0.07	0.07
TCR 02-01-01	3.24	3.43	3.38	3.15	3.30	TCR 02-01-01	15.81	18.36	20.29	19.83	18.57	TCR 02-01-01	0.07	0.08	0.08	0.07	0.08
TCR 06-01-01	2.79	3.58	3.69	3.25	3.33	TCR 06-01-01	12.30	15.16	17.73	15.81	15.25	TCR 06-01-01	0.07	0.09	0.09	0.08	0.08
CE 02-01	3.28	3.29	2.71	3.19	3.12	CE 02-01	12.50	15.33	14.14	14.86	14.21	CE 02-01	0.07	0.07	0.07	0.07	0.07
Mean	3.18	3.56	3.18	3.36	3.32	Mean	13.51	15.24	15.83	16.13	15.18	Mean	0.07	0.08	0.07	0.07	0.07

4.2.2.4 Collar diameterof*Casuarina equisetifolia* for different treatments

At the end of 3 months in ambient conditions, CE 02-04-03 recorded the highest (3.53) while TCR 06-01-01recorded the lowest values of collar diameter (2.79). TCR 12-02-03 ranked the highest (3.81) while CE 02-01 ranked the lowest in collar diameter (3.29) under ET. However under EC, TCR 06-01-01 ranked the highest (3.69) and CE 02-01 ranked the lowest (2.71). Under EC ET conditions, TCR 12-02-03 shows the highest (4.27) and TCR 02-01-01 shows the lowest collar diameter (3.15). Among the clones TCR 12-02-03 shows good response and among the treatment CC shows good response for collar diameter.

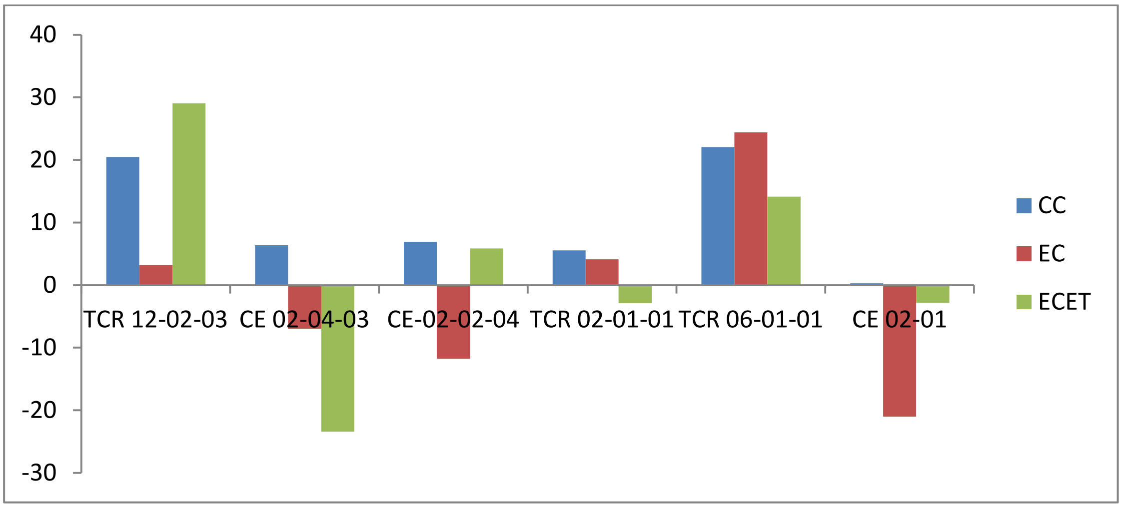

Figure31: Increase or decrease in Collar diameter of *Casuarina equisetifolia* for different treatments (CC, EC and ECET) as percentage over that of ambient condition.

4.2.2.5 Needle lengthof*Casuarina equisetifolia* for different treatments

At the end of 3 months in ambient conditions, TCR 02-01-01 recorded the maximum (15.81) while TCR 12-02-03recorded the minimum value of needle length (12.15). TCR 02-01-01 ranked highest (18.36) andCE 02-04-03 ranked the lowest in needle length (12.81) under ET. However under EC, TCR 02-01-01 ranked the highest (20.29) and CE 02-04-03ranked the lower (13.15). Under EC ET conditions, TCR 02-01-01 shows the highest (19.83) and CE 02-02-04 shows the lowest needle length (14.51). The clone TCR 12-02-03 shows the maximum value of needle length under different conditions. Among the clones TCR 02.01.01shows good response and among the treatment ECET shows good response needle length.

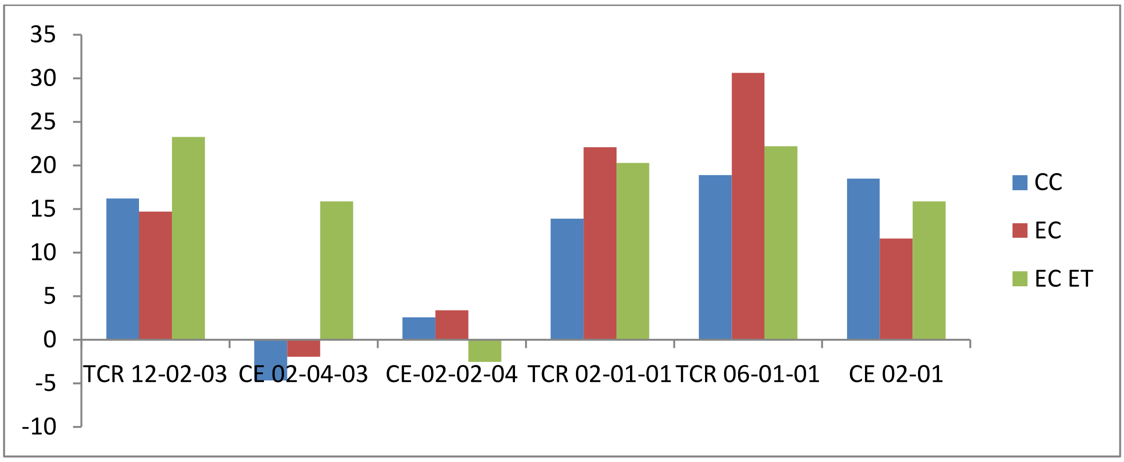

Figure 32: Increase or decrease in Needle length of *Casuarina equisetifolia* for different treatments (CC, EC and ECET) as percentage over that of ambient condition.

4.2.2.6 Needle diameterof*Casuarina equisetifolia* for different treatments

At the end of 3 months in ambient conditions,TCR 12-02-03, TCR 02-01-01, TCR 06-01-01 and CE 02-01 recorded the maximum (0.07) while CE 02-04-03 and CE 02-02-04 recorded the minimum needle diameter (0.06). Under ET condition, the highest needle diameter was shown by TCR 06-01-01(0.09) and all the remaining clones shows the lowest value (0.07) except TCR 02-01-01. However under EC, TCR 06-01-01 ranked the highest (0.09), while TCR 12-02-03 and CE 02-04-03 rankedthe lowest in needle diameter (0.07). Under ECET condition, TCR 12-02-03 and TCR 06-01-01 shows the highest needle diameter (0.08) whereas all the remaining clones shows the lowest value (0.07). Among the clones TCR 02.01.01shows good response and among the treatment CC shows good response needle diameter.

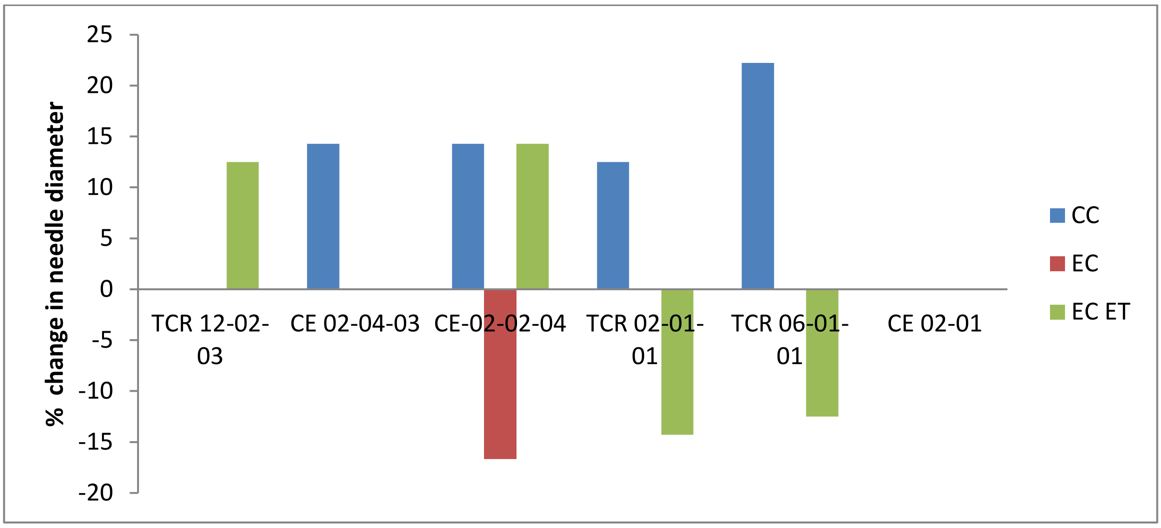

Figure 33: Increase or decrease in Needle diameter of *Casuarina equisetifolia* for different treatments (CC, EC and ECET) as percentage over that of ambient condition.

Table 22: Differential growth response (leaf area and stem volume index) of clones of *Casuarina equisetifolia* to elevated CO_2

Clones	Leaf area					Clones	Stem Volume Index				
	Amb	CC	EC	ECET	Mean		Amb	CC	EC	ECET	Mean
TCR 12-02-03	2.73	3.45	3.07	3.77	3.26	TCR 12-02-03	2.12	3.09	2.04	4.9	3.04
CE 02-04-03	2.54	2.7	3.03	3.19	2.87	CE 02-04-03	2.62	3.96	2.5	2.98	3.02
CE 02-02-04	2.53	3.26	2.76	2.75	2.83	CE 02-02-04	2.95	3.73	4.36	4.01	3.76
TCR 02-01-01	3.29	4.53	4.61	4.49	4.23	TCR 02-01-01	1.8	2.58	1.84	2.28	2.13
TCR 06-01-01	2.49	4.28	4.55	3.97	3.82	TCR 06-01-01	1.87	2.56	2.72	2.03	2.30
CE 02-01	2.73	3.37	2.99	3.33	3.11	CE 02-01	1.76	1.99	1.97	2.02	1.94
Mean	2.72	3.60	3.50	3.58	3.35	Mean	2.19	2.99	2.57	3.04	2.70

4.2.2.7 Leaf areaof*Casuarina equisetifolia* for different treatments

TCR 02-01-01 recorded the maximum leaf area under all treatments. But TCR 06-01-01 shows the lowest (2.49) under ambient conditions, CE 02-04-03 recorded its lowest in ET (2.70) and CE 02-02-04 recorded its lowest values in EC and ECET. Among the clones TCR 02.01.01shows good response and among the treatment CC shows good response leaf area.

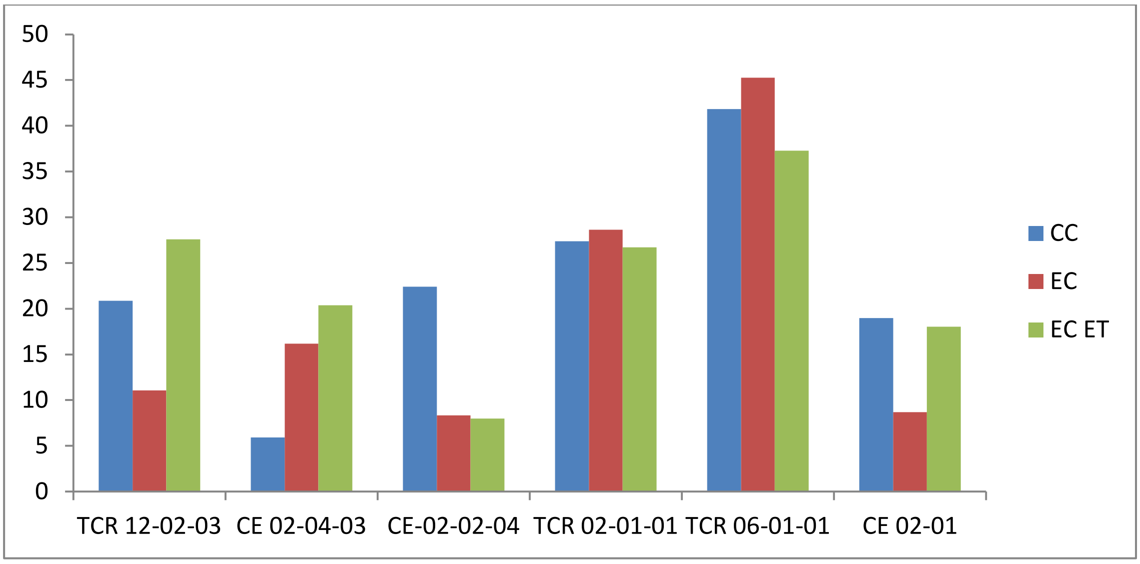

Figure 34: Increase or decrease in Leaf area of *Casuarina equisetifolia* for different treatments (CC, EC and ECET) as percentage over that of ambient condition.

4.2.2.8 Stem Volume Indexof*Casuarina equisetifolia* for different treatments

At the end of 3 months in ambient conditions, CE 02-02-04 recorded the maximum (2.95) while CE 02-01 recorded the minimum SVI (1.76). CE 02-04-03 ranked the highest (3.96) andCE 02-01ranked the lowest in SVI (1.99) under ET. However under EC, CE 02-02-04 ranked the highest (4.36) and TCR 02-01-01 ranked the lower (1.84). Under EC ET conditions, TCR 12-02-03 shows the highest (4.90) and CE 02-01 shows the lowest SVI (2.02). Among the clones C E 02-02-04 shows good response and among the treatment ECET shows good responsestem volume index.

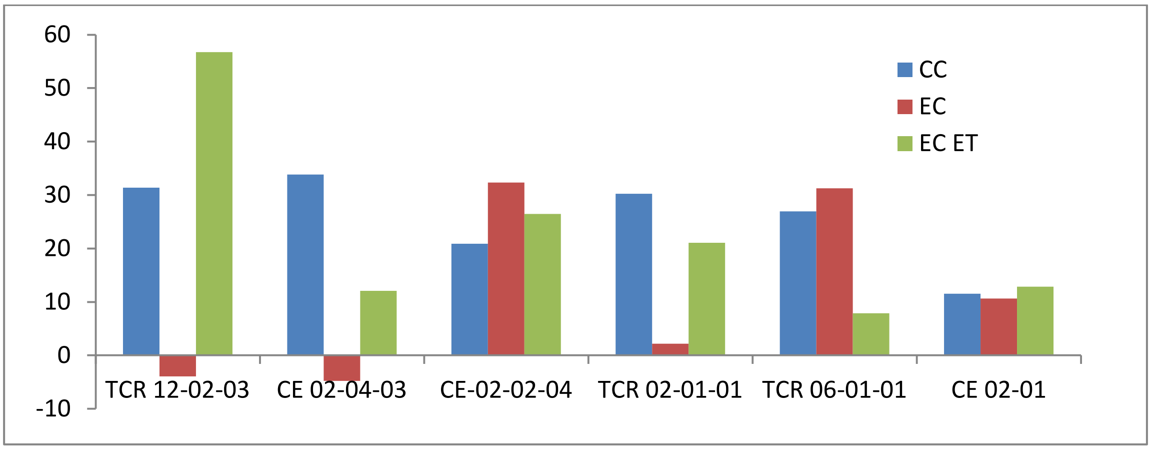

Figure 35: Increase or decrease in Stem Volume Index of *Casuarina equisetifolia* for different treatments (CC, EC and ECET) as percentage over that of ambient condition.

With reference to intra-specific clonal variation in tree species, it was observed that huge variation existed among the clones in terms of total dry matter accumulation in all the three species (Casuarina, Neem and Ailanthus).

Riikonen et al (2003) also reported similar observations, in two clones of silver birch (*Betulapendula*) responded differently to elevatedCO_2 levels.In one clone of silver he observed 40per centvariation under elevated CO_2, but in the other clone there was no visible response.

Similar intra – specific clonal variations have also been reported in Sitka spruse (Murray *et al.,*1994), poplar (Ceulemans *et al*., 1995),*Heveabrasiliensis* (Devakumar *et al*.,1998), and *Poplustremuloids* (Isebrands *et al*., 2003).

Table 23: Differential growth response (fresh weight of root, shoot and leaf) of clones of Casuarina *equisetifolia* to elevated CO_2

Clones	FWR					Clones	FWS					Clones	FWL				
	Amb	CC	EC	ECET	Mean		Amb	CC	EC	EC ET	Mean		Amb	CC	EC	EC ET	Mean
TCR 12-02-03	1.80	2.20	1.14	2.76	1.98	TCR 12-02-03	0.65	0.82	0.73	1.20	0.85	TCR 12-02-03	2.21	2.42	1.99	3.53	2.54
CE 02-04-03	2.16	1.83	2.31	2.26	2.14	CE 02-04-03	1.11	1.38	0.91	0.95	1.09	CE 02-04-03	3.33	3.57	2.58	3.01	3.12
CE 02-02-04	2.25	1.48	2.31	2.20	2.06	CE- 02-02-04	0.95	1.31	1.00	1.26	1.13	CE 02-02-04	3.56	4.06	3.65	3.62	3.72
TCR 02-01-01	2.32	1.32	3.53	1.51	2.17	TCR 02-01-01	0.69	0.77	1.89	1.02	1.09	TCR 02-01-01	2.04	2.60	4.40	3.05	3.02
TCR 06-01-01	2.26	1.84	2.48	2.21	2.20	TCR 06-01-01	0.69	0.78	0.67	0.90	0.76	TCR 06-01-01	1.96	2.49	2.31	2.99	2.44
CE 02-01	0.92	0.63	0.66	1.04	0.81	CE 02-01	0.61	0.66	0.54	0.80	0.65	CE 02-01	2.02	1.99	2.34	2.81	2.29
Mean	1.95	1.55	2.07	2.00	1.89	Mean	0.78	0.95	0.96	1.02	0.93	Mean	2.52	2.86	2.88	3.17	2.86

4.2.2.9 Fresh weightof*Casuarina equisetifolia* for different treatments

Clone CE 02-01 recorded the lowest RFW (0.92) under ambient conditions, while TCR 02-01-01recorded the highest (2.32). The highest RFW was recorded in TCR 12-02-03 (2.20g) under ET, TCR 02-01-01 under EC (3.53) and TCR 12-02-03 under ECET conditions (2.76). Lowest RDW was recorded by CE 02-01 under ET (0.63), EC (0.66), and ECET (1.04) conditions. Among the clones TCR 06-01-01 shows good response and among the treatment EC shows good response RFW.

The highest value of SFW was recorded by the clone CE 02-04-03 under ambient (1.11) and ET conditions (1.38), TCR 02-01-01 under EC (1.89) and by CE 02-02-04 under ECET (1.26) conditions. The lowest SDW was recorded by CE 02-01 under ambient (0.61), ET (0.66), EC (0.54), and ECET (0.80) conditions. Among the clones TCR 02-02-04 shows good response and among the treatment ECET shows good response for SFW.

Underambient, ET and ECET conditions highest LFW was recorded by CE 02-02-04 (3.56g, 4.06g and 3.62g respectively). Under EC, TCR 02-01-01 shows the highest value (4.40g). Under ambient conditions, TCR 06-01-01 (1.96g) recorded lowest value of LFW. TCR 12-02-03 under ET (2.42g), EC (1.99 g) and CE 02-01 under ECET (2.81g) recorded the lowest value. Among the clones CE 02-02-04 shows good response and among the treatment ECET shows good response for LFW.

Table 24: Differential growth response (dry weight of root, shoot, leaf) of clones of *Casuarina equisetifolia* to elevated CO_2

Clones	DWR					Clones	DWS					Clones	DWL				
	Amb	CC	EC	ECET	Mean		Amb	CC	EC	EC ET	Mean		Amb	CC	EC	EC ET	Mean
TCR 12-02-03	0.51	0.82	0.51	0.8	0.66	TCR 12-02-03	0.30	0.39	0.30	0.51	0.38	TCR 12-02-03	1.38	0.90	0.67	1.14	1.02
CE 02-04-03	0.95	0.88	0.76	0.85	0.86	CE 02-04-03	0.57	0.71	0.39	0.43	0.53	CE 02-04-03	1.31	1.3	0.90	1.16	1.17
CE 02-02-04	0.78	0.84	0.75	0.8	0.79	CE- 02-02-04	0.49	0.68	0.48	0.75	0.60	CE 02-02-04	1.40	1.52	1.28	1.37	1.39
TCR 02-01-01	0.80	0.49	1.25	0.64	0.80	TCR 02-01-01	0.27	0.31	0.78	0.41	0.44	TCR 02-01-01	0.67	0.88	1.60	1.10	1.06
TCR 06-01-01	0.73	0.76	0.98	0.93	0.85	TCR 06-01-01	0.34	0.38	0.41	0.44	0.39	TCR 06-01-01	0.71	0.96	0.91	1.13	0.93
CE 02-01	0.39	0.32	0.35	0.36	0.36	CE 02-01	0.29	0.27	0.27	0.35	0.30	CE 02-01	0.8	0.68	0.80	0.91	0.80
Mean	0.69	0.69	0.77	0.73	0.72	Mean	0.38	0.46	0.44	0.48	0.44	Mean	1.05	1.04	1.03	1.14	1.06

4.2.2.10. Dry weightof*Casuarina equisetifolia* for different treatments

The highest RDW was recorded by the clone CE 02-04-03 under ambient (0.95) and ET (0.88) conditions; TCR 02-01-01under EC (1.25), and by TCR 06-01-01 under ECET (0.93) conditions. CE 02-01 recorded the lowest values under all conditions (ambient (0.39), ET (0.32), EC (0.35) and EC ET (0.36)). Among the clones C E 02-04-03 shows good response and among the treatment EC shows good response for RDW.

Under ambient and ET conditions, CE 02-04-03 recorded the highest value of SDW (0.57 and 0.71 respectively). Under EC, the highest value was seen in TCR 02-01-01 (0.78g) and under ECET; CE 02-02-04 (0.75) shows the highestSDW. CE 02-04-03 under ambient (0.27) and CE 02-01under ET (0.27), EC (0.27), EC ET (0.35) recorded the lowestSDW values. Among the clones CE 02-02-04 shows good response and among the treatment ECET shows good response for SDW.

Under ambient and ET conditions, CE 02-02-04 recorded the highest value of LDW (1.40 and 1.52 respectively). The Lowest value was recorded by TCR 02-01-01under ambient (0.67) and CE 02-01under ET conditions (0.68). Under EC condition, TCR 02-01-01 recorded the highest (1.60) while TCR 12-02-03 (0.67) recorded the lowest LDW. Under EC ET, CE 02-02-04 (1.37) and CE 02-01 (0.91) recorded the highest and lowest values respectively. Among the clones CE 02-02-04 shows good response and among the treatment ECET shows good response for LDW.

Table 25: Differential growth response (total fresh weight, total dry weight, MC) of clones of Casuarina equisetifolia to elevated CO_2

Clones	FWT					Clones	DWT					Clones	MC				
	Amb	CC	EC	ECET	Mean		Amb	CC	EC	ECET	Mean		Amb	CC	EC	EC ET	Mean
TCR 12-02-03	4.65	5.43	3.86	7.49	5.36	TCR 12-02-03	2.19	2.1	1.48	2.45	2.06	TCR 12-02-03	52.9	61.33	61.66	67.29	60.80
CE 02-04-03	6.6	6.77	5.79	6.21	6.34	CE 02-04-03	2.82	2.88	2.04	2.43	2.54	CE 02-04-03	57.27	57.46	64.77	60.87	60.09
CE 02-02-04	6.75	6.85	6.97	7.08	6.91	CE 02-02-04	2.67	3.04	2.51	2.93	2.79	CE 02-02-04	60.44	55.62	63.99	58.62	59.67
TCR 02-01-01	5.05	4.7	9.81	5.57	6.28	TCR 02-01-01	1.74	1.68	3.63	2.16	2.30	TCR 02-01-01	65.54	64.26	63	61.22	63.51
TCR 06-01-01	4.90	5.10	5.45	6.10	5.39	TCR 06-01-01	1.77	2.10	2.3	2.5	2.17	TCR 06-01-01	63.88	58.82	57.8	59.02	59.88
CE 02-01	3.55	3.27	3.53	4.65	3.75	CE 02-01	1.48	1.26	1.42	1.63	1.45	CE 02-01	58.31	61.47	59.77	64.95	61.13
Mean	5.25	5.35	5.90	6.18	5.67	Mean	2.11	2.18	2.23	2.35	2.22	Mean	59.72	59.83	61.83	62.00	60.84

4.2.2.11 Total Fresh weightof*Casuarina equisetifolia* for different treatments

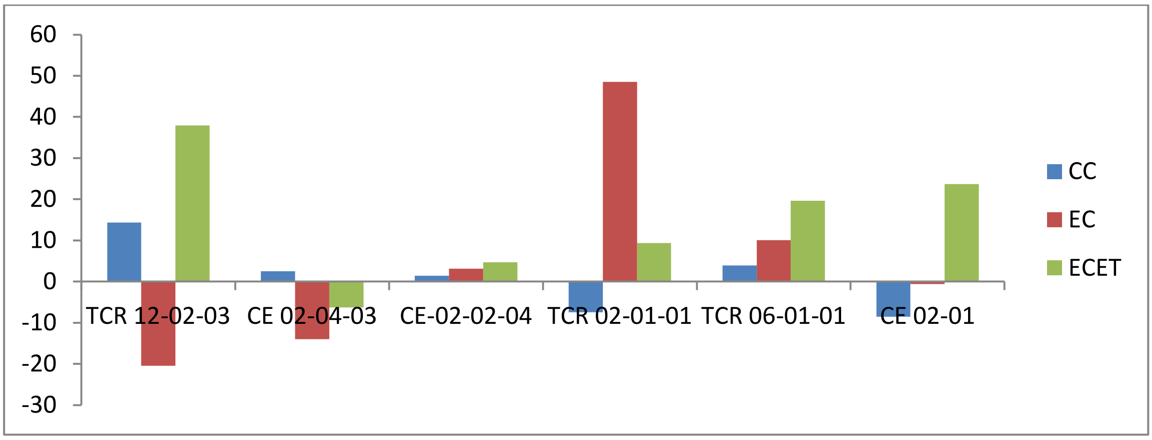

Figure 36: Increase or decrease in Total fresh weight of *Casuarina equisetifolia* for different treatments (CC, EC and ECET) as percentage over that of ambient condition.

Clone TCR 02-01-01shows the maximum fresh weight (9.81) in EC condition followed by TCR 12-02-03 (7.49). CE 02-02-04showsa higher value (6.85) in ET while CE 02-02-04recorded almost similar value (6.75) in ambient conditions. The lowest fresh weight values were recorded in CE 02-01 clone (3.27) in all treatments. (Table18). CE 02-02-04 shows good response and among the treatment ECET shows good response total fresh weight.

4.2.2.12 Total dry weightof*Casuarina equisetifolia* for different treatments.

At the end of 3 months in ambient condition TCR 02-01-01 recorded maximum value (3.63) whileCE 02-01 recorded minimum value of TDW (1.26). CE 02-04-03 ranked the highest (2.82) andCE 02-01 rankedthe lowest (1.48) under ambient conditions. However under ET, CE 02-02-04 ranked the highest (3.04) and CE 02-01 ranked the lowest (1.26) in TDW. TCR 02-01-01 shows the highest (3.63) and CE 02-01 shows the lowest dry weight (1.42) under EC condition. CE 02-02-04 (2.93) and CE 02-01(1.63) recorded the maximum and minimum values of TDW under EC ET condition respectively. Among the clones CE 02-02-04 shows good response and among the treatment ECET shows good response for total dry weight. Greater variation existed in dry matter accumulation in biomass among different clones under elevated CO_2 conditions. (Buvaneswaran *et al*., 2016).

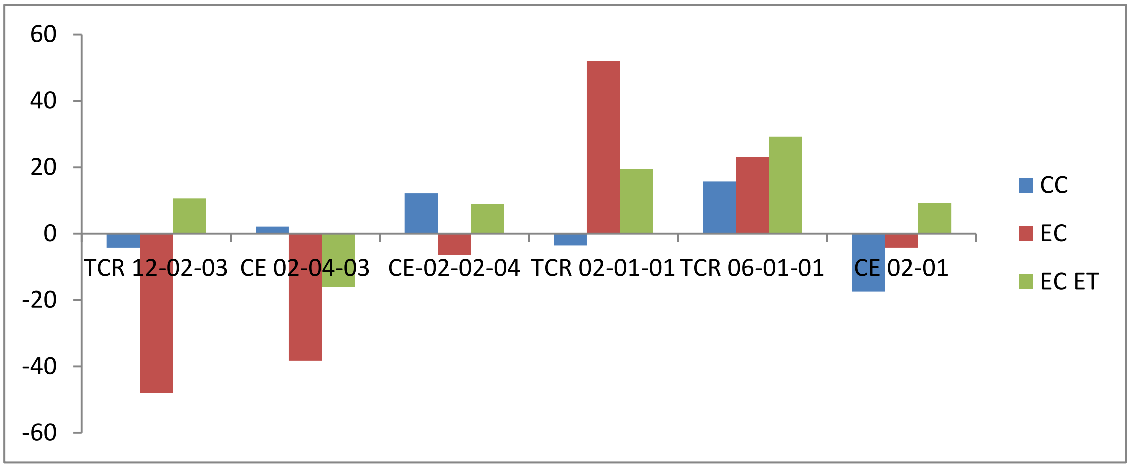

Figure 37: Increase or decrease in Total dry weight of *Casuarina equisetifolia* for different treatments (CC, EC and ECET) as percentage over that of ambient condition.

Table 26: Differential growth response (protein, phenol) of clones of *Casuarina equisetifolia* to elevated CO_2

Clones	Proteins					Clones	Phenols				
	Amb	CC	EC	ECET	Mean		Amb	CC	EC	ECET	Mean
TCR 12-02-03	52.22	53.89	45.59	51.26	50.74	TCR 12-02-03	4.24	2.72	4.92	3.95	3.96
CE 02-04-03	152.11	90.36	137.26	101.01	120.19	CE 02-04-03	10.07	6.25	8.7	9.05	8.52
CE 02-02-04	188.36	183.82	192.72	179.45	186.09	CE 02-02-04	14.02	11.16	13.01	13.84	13.01
TCR 02-01-01	66.66	58.88	67	63.72	64.07	TCR 02-01-01	5.84	3.76	5.04	5.19	4.96
TCR 06-01-01	71.96	63.65	44.49	55.04	58.79	TCR 06-01-01	5.75	3.38	3.29	4.58	4.25
CE 02-01	96.96	59.31	56.89	68.95	70.53	CE 02-01	6.02	2.3	4.79	2.88	4.00
Mean	104.71	84.99	90.66	86.57	91.73	Mean	7.66	4.93	6.63	6.58	6.45

4.2.2.13 Proteinsof*Casuarina equisetifolia* for different treatments

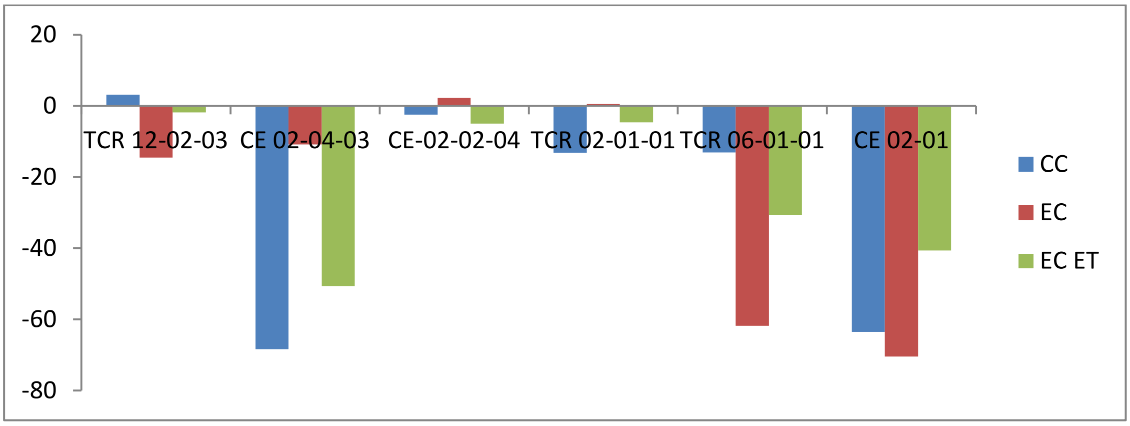

Figure 38: Increase or decrease in Proteins of *Casuarina equisetifolia* for different treatments (CC, EC and ECET) as percentage over that of ambient condition.

At the end of 3 months in ambient conditions, CE 02-02-04 recorded the maximum (188.36) and TCR 12-02-03recorded minimum protein content (52.22). MTP 27 ranked the highest (183.82) while CJ6 ranked lowest in protein content (53.89) under ET. However under EC, MTP 27 ranked the highest (192.72) and CJ8 ranked the lowest (44.49). Under EC ET condition, MTP 27 shows the highest (179.45) and CJ8 shows the lowest protein content (51.26). Among the clones CE 02-02-04 shows good response and among the treatment Ambient shows good response for proteins.

4.2.2.14 Phenolsof*Casuarina equisetifolia* for different treatments

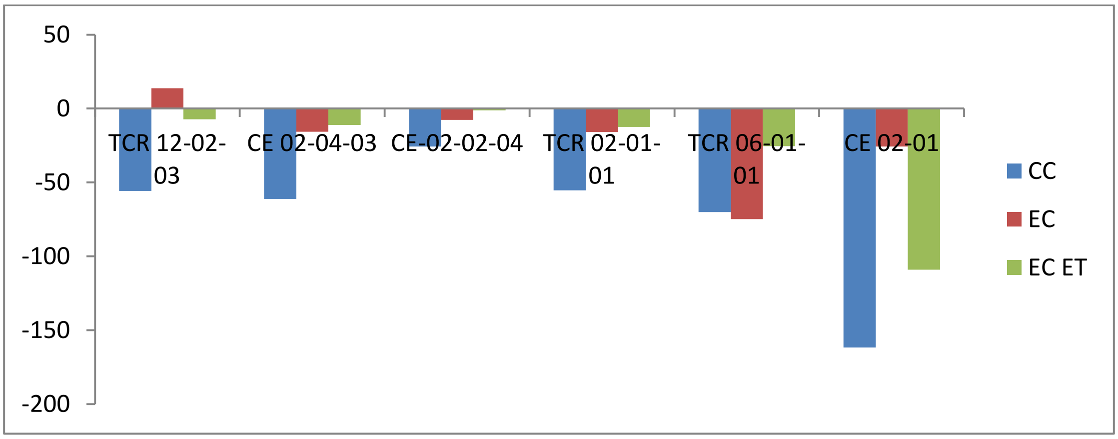

Figure 39: Increase or decrease in Phenols of *Casuarina equisetifolia* grown for different treatments (CC, EC and ECET) as percentage over that of ambient condition.

At the end of 3 months in ambient condition, MTP 27 recorded the maximum (14.02) and CJ8recorded the minimum phenol content (4.24). MTP 27 ranked the highest (11.16) while CJ6 was the lowest in phenol content (2.72) under ET. However under EC, CJ12 ranked the highest (13.01) and CJ8 ranked the lowest (3.29). Under EC ET conditionCJ17 shows the highest (13.84) and CJ18 shows the lowest phenolcontent (2.88). Among the clones CE 02-02-04 shows good response and among the treatment ambient shows good response for phenols.

Table 27: Differential growth response (carbohydrate and reducing sugar) of clones of *Casuarina equisetifolia* to elevated CO_2

Clones	Carbohydrates				Mean	Clones	Reducing Sugars				Mean
TCR 12-02-03	37.19	45.3	66.1	58.08	51.67	TCR 12-02-03	388.33	404.87	387.81	422.65	400.92
CE 02-04-03	61.16	43.07	77.55	51.96	58.44	CE 02-04-03	451.24	434.83	399.01	433.47	429.64
CE 02-02-04	59.00	42.08	89.36	72.94	65.85	CE 02-02-04	448.45	420.93	406.28	416.51	423.04
TCR 02-01-01	74.44	52.46	92.26	52.94	68.06	TCR 02-01-01	403.30	422.65	405.03	414.43	411.35
TCR 06-01-01	61.45	63.18	73.61	58.41	64.16	TCR 06-01-01	405.06	418.12	398.26	408.36	407.45
CE 02-01	51.02	48.54	72.42	67.88	59.97	CE 02-01	441.68	383.25	362.54	375.41	390.72
Mean	57.30	49.1	78.50	60.30	61.35	Mean	423.01	414.10	393.10	411.80	410.50

4.2.2.15 CarbohydratesofCasuarina equisetifolia for different treatments

At the end of 3 months in ambient conditions, CJ17recorded the maximum (74.44) while CJ12 recorded minimum carbohydrate content (37.19). MTP 27 ranked the highest (63.18) while CJ12was again rankedthe lowest (42.08) under ET. However under EC, CJ18ranked the highest (92.26) and CJ6 recorded the lowest carbohydrate content (66.10). Under EC ET condition, MTP 27 shows the highest (72.94) and CJ18 shows the lowest carbohydrate content (51.96). Among the clones TCR 02-01-01 shows good response and among the treatment EC shows good response for carbohydrates.

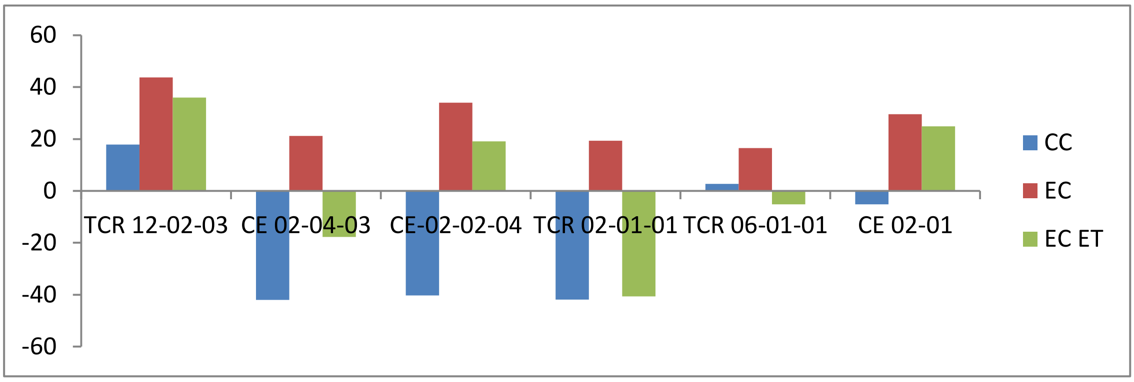

Figure 40: Increase or decrease in Carbohydrate of *Casuarina equisetifolia* for different treatments (CC, EC, and ECET) as percentage over that of ambient condition.

4.2.2.16 Reducing sugarof*Casuarina equisetifolia* for different treatments

At the end of 3 months in ambient conditions, CJ 17 recorded the maximum (451.24) whileCJ8recorded minimum value of reducing sugars (403.30). CJ17ranked the highest (434.83) while CJ8 ranked the lowest (383.25) under ET. However under EC, CJ17 ranked the highest (399.01) and CJ8 the lowest (362.54). Under EC ET condition CJ17 shows the highest (433.47) and CJ8 shows the lowest reducing sugar contentAmbient shows good response (375.41).Among the clones CE 02.04.03 shows good response and among the treatment Ambient shows good response for reducing sugar.

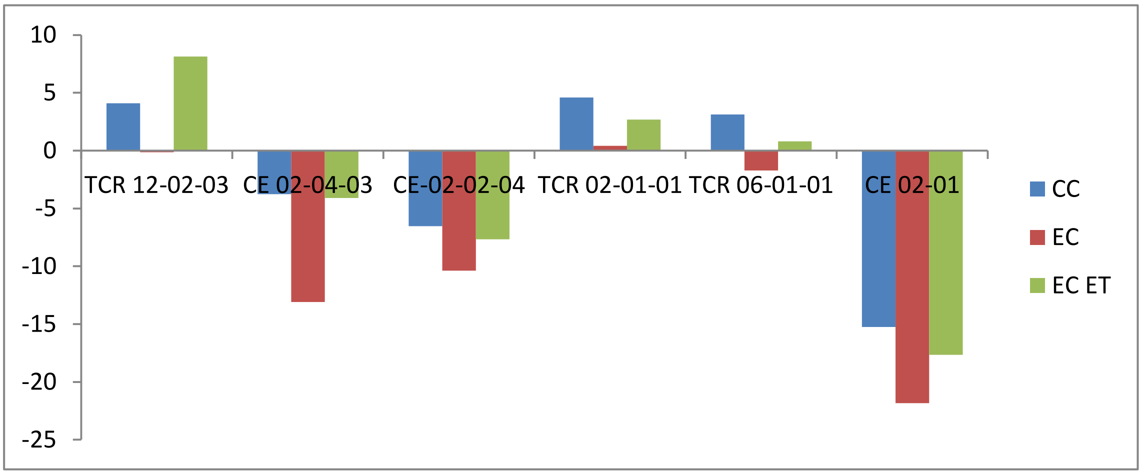

Figure 41: Increase or decrease in reducing sugars of *Casuarina equisetifolia* for different treatments (CC, EC, and ECET) as percentage over that of ambient condition.

4.2.2.17 Total Chlorophyllof*Casuarina equisetifolia* for different treatments

At the end of 3 months in ambient conditions, TCR 06-01-01 recorded the maximum (18.31) while CE 02-01recorded the minimum value of total chlorophyll (14.11). TCR 06-01-01 ranked the highest (21.03) while CE 02-01 was again the lowest (11.03) under ET. Under EC, TCR 06-01-01 ranked the highest (21.45) and CE 02-01 again rankedthe lowest (9.65). TCR 12-02-03 shows the highest (16.82) and CE 02-01 shows the lowest total chlorophyll content (10.67) under EC ET condition. Among the clones TCR 12-02-03shows good response and among the treatment CC shows good response for total chlorophyll.

Table 28: Differential growth response (total chlorophyll) of clones of *Casuarina equisetifolia* to elevated CO_2

Clones	Total chlorophyll				
	Amb	CC	EC	ECET	Average
TCR 12-02-03	17.45	18.54	15.93	16.82	17.19
CE 02-04-03	17.31	16.05	15.70	12.59	15.41
CE-02-02-04	13.39	15.58	15.46	12.53	14.24
TCR 02-01-01	15.13	16.09	16.84	12.16	15.06
TCR 06-01-01	18.31	21.03	21.45	15.61	19.10
CE 02-01	14.11	11.03	9.65	10.67	11.37
Mean	15.95	16.39	15.84	13.40	15.39

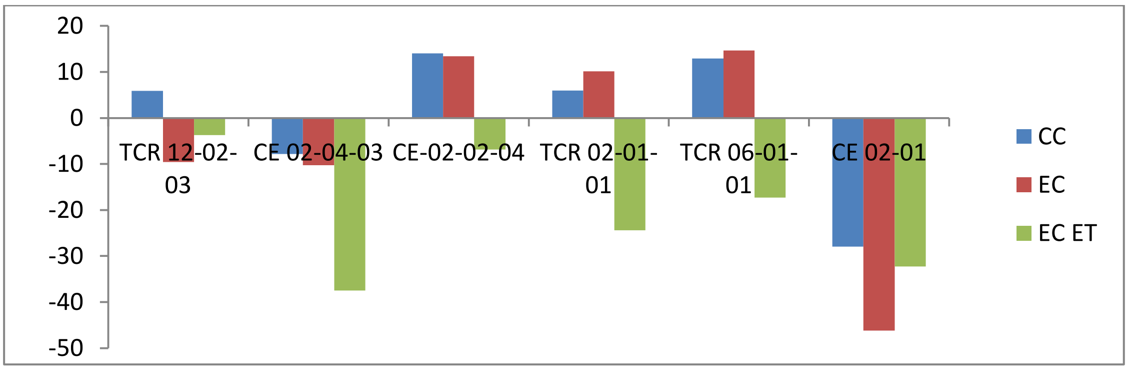

Figure 42: Increase or decrease in Total chlorophyll of *Casuarina equisetifolia* for different treatments (CC, EC, and ECET) as percentage over that of ambient condition.

Among the species *Casuarina equisetifolia* CE 02.02.03 and TCR 02.01.01 shows better performance under ambient conditions, but under ET condition CE 02.04.03 and CE 02.02.04 performed better. Under ECO_2 condition, TCR 02.01.01 and CE 02.02.04 and under ECET, CE 02.02.04 and TCR 12.02.03shows better growth. From these results it was concluded that CE 02.02.04 was better clone suitable forfuture climate.

Based on the three months data was concluded that, MTP 27 and CE 02.02.04 will perform better under future climatic conditions. The more responsive clones are more adaptive to the future conditions and this data will be helpful in planning future agro forestry/afforestation programs and plantation technique.

Table 29: Correlation coefficient matrix for more responsive parameters 10 components in different levels of CO_2 exposure in clones of Casuarina

	Shoot Length	RDW	RFW	Needle Length	Leaf area	Protein	Phenol	Carbohydrate	Reducing sugar	Total chlorophyll
Shoot length	1.00									
RDW	.515**	1.00								
RFW	.440**	.904**	1.00							
Needle length	0.02	-.153**	-.191**	1						
Leaf area	.123*	0.112	0.087	.695**	1					
Protein	0.072	0.044	0.025	-0.011	-.175**	1				
Phenol	.305**	.440**	.391**	-.166**	-0.087	.722**	1			
Carbohydrate	.479**	.624**	.617**	-.123*	0.099	0.038	.331**	1		
Reducing sugar	.344**	.531**	.490**	-.266**	-0.066	.165**	.471**	.402**	1	
Total Chlorophyll	-.156**	-.270**	-.253**	0.092	-0.023	-.183**	-.368**	-.401**	-.155**	1

**Correlation is significant at the 0.01 per cent level (2tailed).

* Correlation is significant at the 0.05 per cent level (2-tailed).

4.5 CORRELATION STUDY

The correlation coefficient matrix was developed for 10 top contributing parameters. Simple correlations presented in Table 29 depict the correlation existing between the biometric and biochemical parameters.

- Plant shoot length of plants was positively correlated to phenols, carbohydrate content and reducing sugar but negatively correlated to chlorophyll b, total chlorophyll and chlorophyll A/B ratio.
- The case was also same with RDW and SDW. Needle length was negatively correlated to phenols, carbohydrate content and reducing sugars.
- Leaf area was negatively correlated to proteins.

CHAPTER 5. SUMMARY AND CONCLUSION

An experiment was conducted at Institute of Forest Genetics and Tree Breeding Coimbatore, Tamil Nadu to study the morphological and biochemical response of Casuarinaunder elevatedCO_2 conditions, and also to find the inter and intra specific variations of the crop under elevated CO_2.The observations on morphological and biochemical parameters were recorded for three months. The results of the study show the actual response of Casuarina under future climatic conditions. The summary of the findings of the present study are presented below.

5.1 MORPHOLOGICAL AND BIOCHEMICAL RESPONSES OF CASUARINA TO ELEVATED CO_2

Elevated CO_2 had a significant positive effect on plant height, leaf area and stem volume index than other conditions. Biomass production under EC condition was approximately similar to that of ambient conditions.

Protein levels in the ambient and EC clones were relatively higher than that of ET and EC ET due to heat stress. Phenols were the highest in ambient plants with further decrease in ET plants as heat stress reduced the phenolic content of the plants. EC was found to be a reducer of phenolic content, which was evident from EC and EC ET treatments. Among the elevated CO_2plants, the combination of heat stress further reduced the phenolic content in those plants. Due to CO_2 fertilization, the amount of carbohydrates produced in the elevated CO_2plants were higher, when compared to control plants, with only EC plants showing the highest carbohydrate production as the production of carbohydrates were hampered by the heat stress induced in the EC ET plants. Due to the heat stress, the ET and EC ET produced higher amount of reducing sugars for combating the reactive oxygen species which arose due to the very stress.Both *Casuarina equisetifolia* and *Casuarina junghuhniana*, positively responded even at 900 ppm of CO_2 elevation and these species may be considered for greater carbon sequestration under elevated CO_2 and temperature levels. (*Buvaneswaran et al.*, 2018)

5.2 INTER AND INTRA SPECIFIC VARIATIONS INCASUARINATO ELEVATED CO_2

There is no significant difference between the *Casuarina equisetifolia* and *Casuarina junghuhniana.* But within each species there were noticeable variations. It was observed that all parameters except leaf area were significant with respect to days of the treatment. CO_2 levels were significant at $p < .01$ level in needle diameter and $p<.001$ level in collar diameter. Clonal variations were significant at $p < .001$ level in all parameters except leaf area. The interaction effect of treatments and clones were significant at .01 levels at root length and .001 significant in needle diameter, collar diameter and stem volume index. The results also shows that CO_2 levels were significant at $p < .05$ level in fresh weight and $p<.001$ level in RFW and Fresh Weight of RSR. Clonal variations were significant at $p < .001$ level for all parameters. The interaction effect of treatments and clones were significant at 0.01 levels at RFW and SFW and 0.001 significant RFW, RSR and TFW.

In the species *Casuarina* with reference to total dry matter production, CJ18 recorded greater accumulation of biomass under elevated CO_2 and also under ECET condition followed by MTP27. Greater biomass accumulation under elevated $CO_2$and temperature also implies greater carbon sequestration potential of CJ 18 and MTP 27 clones. CJ 18 and MTP 27.Hence, these two clones may be better suited to future climate.

In the species *Casuarina equisetifolia* CE 02.02.03 and TCR 02.01.01 shows better performance under ambient condition, but under ET condition CE 02.04.03 and CE 02.02.04 were better performers. Under EC condition TCR 02.01.01, CE 02.02.04 and under ECET, CE 02.02.04 and TCR 12.02.03 show better growth. From these results it isconcluded that CE 02.02.04 is suitable for the future climate.

Based on three months data it was concluded that CJ18 and MTP 27 in *Casuarina junghuhniana* and CE 02.02.04 and TCR 02.01.01 in *Casuarina equisetifolia* will perform better under future climatic conditions. The more responsive clones will be more adaptive to the future climatic conditions and this data will helpful for future agro forestry/ afforestation programs and plantation techniques.

CHAPTER 6. REFERENCE

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INTER AND INTRA SPECIFIC VARIATIONS OF CASUARINA UNDER ELEVATED CO_2

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ABSTRACT OF THE THESIS

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ABSTRACT

The research was conducted Open Top Chamber facility of Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore from 1st August to 28th October 2017 to study inter and intra specific variations of *Casuarina equisetifolia* and *Casuarina junghuhniana* under projected climate variables (elevated CO_2 and temperature). Six clones of each species were used to study the intra-specific variations. *Casuarina equisetifolia* and *Casuarina junghuhniana* clones were exposed to Ambient (400ppm of CO_2), ET (Chamber control- natural elevation of temperature due to chamber effect, EC (800 ppm of CO_2 with temperature reduced using humidifier), and ECET (800 ppm of CO_2 with natural elevation of temperature) under Open Top Chambers (OTCs). The clones under EC had a significant positive effect on plant height, leaf area and stem volume index than other conditions. The amounts of protein present in the ambient and EC were relatively higher than ET and ECET which were subjected to heat stress. Phenols were the highest in ambient plants with further decrease in ET plants as heat stress reduces the phenol content of the plants. ElevatedCO_2 is also a reducer of phenol content. In EC ET the combination heat stress further reduces the phenol content in the plants. Due to CO_2 fertilization the amount of carbohydrates produced in the elevated CO_2 plants are higher, when compared to control plants, with only EC plants showing the highest carbohydrate production as the production of carbohydrates were hampered by the heat stress induced in the EC ET plants. Due to the heat stress, the ET and EC ET produced larger amount of reducing sugars for combating the reactive oxygen species which arises due to the very stress.

Salient observations of the present study is that there existed intra-specific variations both in *Casuarina junghuhniana* and *Casuarina equisetifolia* in response to elevated CO_2, particularly with reference to dry matter accumulation. Further, among the clones studied, CJ18 and MTP 27 in *Casuarina junghuhniana* and CE 02.02.04 TCR and 02.01.01 in *Casuarina equisetifolia* are recorded as better suited tree

varieties to elevated CO_2 environment. Hence the present study confirms the scope for selection of tree varieties adapted to future climatic condition.