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Effect of Climate Change on Seed Germination and Seedling Attributes of *Calophyllum inophyllum* L.

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Abstract: The acclamatory responses of plants to the changing climate are a subject of debate over the past two decades. Hence, it is prudent to understand the response of tree species in the initial stages, as seed and seedlings, to the elevated CO_2 conditions. The study on seed germination, seedling growth and stomatal density analysis of *Calophyllum inophyllum* L. revealed that, the elevated CO_2 negatively affected the germination of the species. But, after the germination, elevated CO_2 condition positively influenced the seedling height growth. Stomatal density of the seedlings was negatively affected by the elevated CO_2 condition.

Keywords: Climate change, Elevated CO_2, Seed germination, Seedling growth, Stomata

Global climate change induced by the anthropogenic activities is the major challenge faced by the world in this 21st century. Humans are having long-term cumulative impacts on earth's ecosystems through a range of consumptive, exploitive and indirect mechanisms, even to the extent of influencing the global climate (Yumnam and Ronald 2022). According to the IPCC (2015) the total contribution of the agriculture, forestry and other land use systems towards the greenhouse gases emissions was 24 per cent. Moreover, the city transport sector, industrialization and reduction in green space are leading more to climate change (Pradhan et al 2022). Under this situation observing and predicting the forest tree seed germination and seedling growth under elevated CO_2 conditions is an urgent need. Although seed germination is under genetic control (Gutterman 2000, Nordan et al 2009) and parental environment (e.g. availability of light, temperature, water, and nutrients) can significantly influence germination characteristics (Marty and Bassirirad 2014, Maharana Rashmiprava et al 2018, Chauhan et al 2019). Although the evidences in the literature show species specific response of seed germination to elevated CO_2, the data on the major species is lacking. Further, increasing atmospheric carbon dioxide concentration has profound effects on growth and development of tree seedlings. A doubling of CO_2 generally stimulates photosynthesis and can lead to substantial increase in growth. The response of seedlings to elevated CO_2 greatly depend on the nutrient reserves of the soil too. Short-term experiments in tree species exposed to elevated CO_2 levels have shown that increased photosynthetic rate up to 40-80 per cent in *Pinus ponderosa, P. radiata, Quercus coccinea* and *Populus deltoides* seedlings (increased net production by 20 per cent (Couteaux and Bottner 1996). Since the studies on response of seed germination and seedling growth to elevated CO_2 of commercially important seedlings are scanty, it is prudent to understand the response of tree species in the initial stages, as seed and seedlings, to the elevated carbon dioxide conditions from the point of climate change and global warming in the future. Further, the acclamatory responses of seedlings to elevated CO_2 conditions changes stomatal density. The variations in stomatal density are attribute to the total water use efficiency of plants and are major considerations in species recommendations in the future. *Calophyllum inophyllum* L. of family Guttifereae (Clusiaceae) is a tree species native to India, East Africa, South East Asia, Australia and South Pacific and is commonly called as 'Indian laurel'. It is a broad leaved evergreen tree occurring as a littoral species along the beach crests, although sometimes occurring inland and adjacent lowland forest and widely planted throughout the tropics and is naturalized in the main Hawaiian Islands. Oil from the nuts has been traditionally used for medicine and cosmetics and is today being commercially used for the production of biodiesel. Annual yield of 20-100 kg/tree of whole fruits have been reported. The nut kernel contains 50-70 per cent oil and the mature tree may produce 1-10 kg of oil per year depending upon the productivity of the tree and the efficiency of extraction process (Chavan et al 2013). In the present study, an attempt was made to understand the response of *C. inophyllum* seeds and seedlings to the elevated CO_2 condition.

MATERIAL AND METHODS

The experiment was carried out at College of Forestry, Ponnampet, Kodagu, Karnataka. Seeds from matured fruits were collected and subjected to mechanical scarification by removing seed coat by hammer. A polytunnel (10.56 m X 0.71 m X 1.26 m) was erected to create elevated carbon dioxide conditions. The floor of the tunnel was spread with the farm yard manure which on decomposition releases the CO_2 and the quantity of farm yard manure to be added was decided as per the procedure given by Devakumar et al (1996). In all, 800 polythene bags were filled with the potting mixture, one batch having 400 polythene bags (100 polythene bags/replication) was kept in open condition as control, and similarly, another batch with 400 polythene bags (100 bags/replication) was placed in the CO_2 enriched condition. One seed per polythene bag was dibbled under both the conditions. Everyday observation of temperature and CO_2 concentrations in the polytunnel were recorded at 9.30 AM and 4.00 PM using CO_2 analyzer (GC 2028) and monthly average was computed. In the control condition average monthly CO_2 concentration was found to be 401.94 ppm with average temperature 25.25ºC whereas in elevated conditions CO_2 concentrations were elevated to 832.53 ppm with 26.12ºC temperature (Fig. 1).

Observation on seed germination were recorded till the end of germination. Growth parameters like seedling collar diameter and the seedling height were measured at the end of the experiment (6 months). Estimation of variations in stomatal density in the open and elevated CO2 concentrationwas carried out after 90 days of sowing. Every third leaf from the top of the plant was harvested, and its area was determined using the leaf area meter. The selected leaves were applied with a quick fix (glue) at both the surfaces which on drying, the glue containing the epidermal cell was removed to observe under the microscope. The number of stomata in the field of view was counted, and their density was calculated per unit leaf area. The observed parameters were:

Days taken for initiation of germination: Number of days taken to initiate germination in each treatment was recorded.

Germination Energy (G.E.): The per cent of seeds in a given sample that germinated up to the time of peak germination.

Germination: The germination was recorded when cotyledons emerged out from the potting mixture and expressed in percentage.

$$\text{Germination (\%)} = \frac{\text{Number of Seed germinated}}{\text{Number of seeds sown}} \times 100$$

Mean Daily Germination (MDG):

$$\text{Mean daily germination} = \frac{\text{Cumulative per cent germination}}{\text{Total number of days for germination}}$$

Peak Value (PV): Maximum mean daily germination reached at any stage of germination period.

Germination Value (GV): This is calculated by formula given by Czabator (1962)

Germination value (GV) = Average mean daily germination × Germination × Peak value

Stomatal density:

$$\text{Number of stomata per unit leaf area} = \frac{\text{Number of stomata in entire leaf}}{\text{Leaf area}}$$

Response Index (RI):

Response index for all the germination parameters under elevated CO_2 was calculated using the formula (Hegde et al 1993):

$$Response\ Index = \frac{Treatment\ mean - Control\ mean}{Control\ mean} \times 100$$

RESULTS AND DISCUSSION

The seed germination in the open area was 82.50 per cent which was significantly higher than the seed germination under CO_2 elevated condition (49.00 %). A significant negative response index value for the all the germination parameters revealed the existence of the deleterious effect of elevated CO_2 on seed germination. The number of days taken to initiate germination in open condition (22 days) was significantly lesser than the elevated CO_2 condition (27 days). The high positive response index value for the initiation of germination (0.23) indicated that the germination was prolonged under

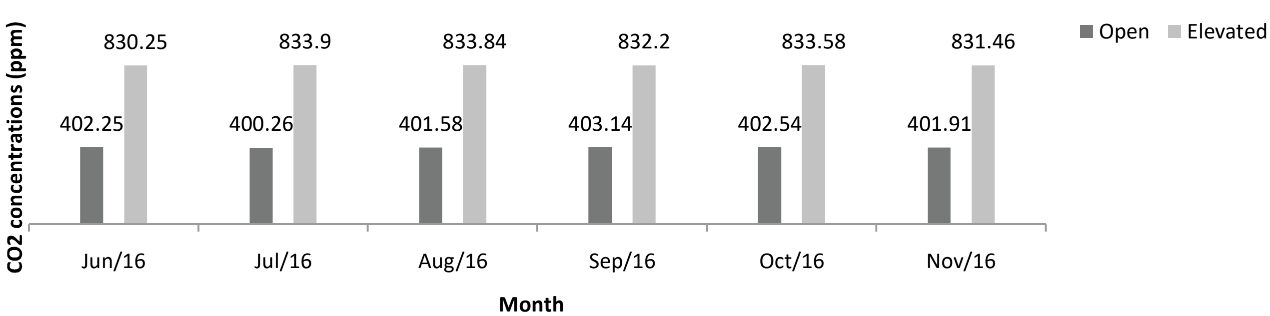

Fig. 1. Mean monthly concentration of CO_2 in open and elevated conditions

elevated CO_2 conditions. Further, the peak value (-0.05), germination value (-0.15) and the mean daily germination (-0.11) had shown a negative response index value depicting the deleterious effect of elevated CO_2 conditions on germination parameters. Germination energy, which needs to be lower for ideal germination showed a high positive response index to elevated condition (0.29) depicting that elevated CO_2 condition has a significant undesirable effect on seed germination of *C. inophyllum*. There was significant increase in the seedling height after six months was observed under the elevated CO_2 condition was indicated by the positive response index value (26.07). In contrast, the collar diameter of the seedlings was negatively affected by the elevated conditions. The study on the stomatal analysis revealed that the stomatal density was reduced under the elevated CO_2 conditions (179.98) than open condition (113.46). However, the results are not significant (Table 1).

Seed germination and speed of germination largely governs plant regeneration from seed. Although seed germination is under genetic control, parental environment can significantly influence germination characteristics. One of the environmental parameters which affect the seed germination and its rate is CO_2 concentration. In the present study, a significant effect of elevated CO_2 on germination of *C. inophyllum* was evident (Table 1). Poor germination of seeds under the elevated CO_2 condition as evidenced by the negative response index could be considered as the negative or deleterious effect of increased CO_2 in the atmosphere (Fig. 2). Seed germination was reduced by 40.6 per cent in elevated CO_2 conditions when compared to open conditions. The elevated CO_2 condition considerably reduced all the germination parameters. The major gases which play a key role in seed germination are ethylene, CO_2 and oxygen. The deprivation of oxygen by increased CO_2 and ethylene coupled with increased temperature has an inhibitive effect on seed germination. Even the higher temperature affects the sensitivity of the seed coat to the surrounding gaseous environment thereby impacting the seed germination negatively (Kigel and Galili 1995). The results were in accordance with the meta analysis carried out by Marty and Bassirirad (2014) on the impact of elevated CO_2 on tree seed germination where percentage germination responses to

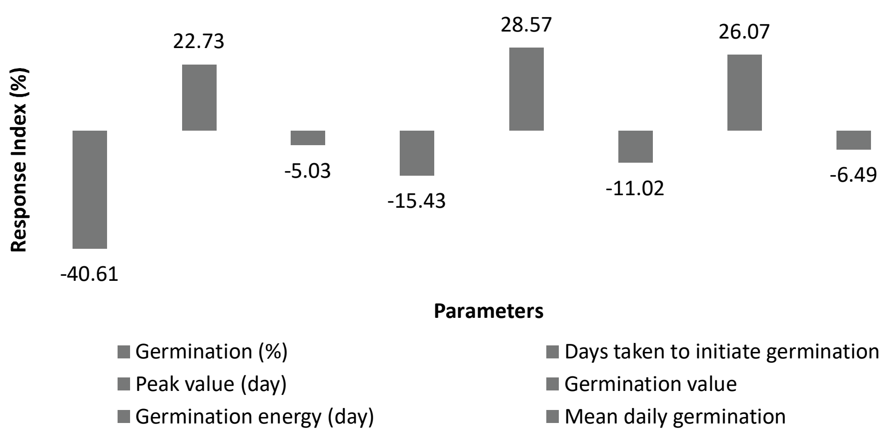

Fig. 2. Response index of the species to elevated CO_2

Table 1. Effect of elevated CO_2 on seed germination and seedling growth

Parameters	Control/Open	Elevated CO_2	t (α=0.05)	p-value
Germination (%)	82.50 (65.27)[*]	49.00 (44.23)	9.10	0.0001
Days taken to initiate germination	22 (4.77)[+]	27 (5.22)	-5.64	0.001
Peak value (day)	1.59	1.51		
Germination value	1.88	1.59		
Germination energy (day)	28	36		
Mean daily germination	1.18	1.05		
Seedling height (cm)	25.47	32.11	-2.72	0.035
Collar diameter (mm)	6.01	5.62	0.74	0.488
Stomatal density	179.98 (13.42)[+]	113.46 (10.65)	2.33	0.066

[*]Values in parenthesis indicate arcsine transformed values; [+]Values indicate square root transformed values

increased CO_2 concentration were highly variable, ranging from a 60 per cent decrease in *Abutilon theophrasti* to a 239 per cent increase in *Pinus taed* (Marty and Bassirirad 2014).

Mean daily germination was also manipulated by the elevated CO_2 (Table 1). Maximum mean daily germination was recorded under open condition (1.18) when compared to elevated conditions (1.05). It may be due to the effect of elevated CO_2 which inhibited the seed respiration (Kigel and Galili 1995) which in turn decreased the mean daily germination (Fig. 1). Time taken to attain peak daily germination is considered as germination energy or energy period. Higher germination energy which is an indicator of slower germination was found to be more for seeds subjected to the elevated CO_2 condition. Thus, carbon dioxide increase in the atmosphere would reduce the rate of germination. Generally, seeds with higher germination and minimum energy period are ideal to produce uniform and quality seedlings. Seeds with longer energy period required more nursery retention time compared to the seeds of shorter energy period (Varsha, 2016). The delayed germination energy in case of elevated conditions could be due to the prolonged initiation of germination in elevated conditions (27 days) when compared to open conditions (22 days). Germination value of the seeds was found to be higher in the open condition than the elevated CO_2 condition. The maximum germination occurred per day (Peak value) was marginally higher at open conditions when compared to elevated CO_2 conditions. Thus, the elevated CO_2 condition has significantly impacted the seed germination and its associated parameters in *C. inophyllum*.

The present study revealed the increased seedling height under elevated CO_2 condition. The elevated CO_2 increase the carboxylation efficiency relative to oxygenation resulting in reduced photorespiration. The increase in seedling height might be due to increased use of CO_2 for carbon assimilation. Evidence from literature showed that it is possible to increase plants height under elevated CO_2concentration (Kumar et al 2001, Salimath et al 2018). But the decrease in the collar diameter of the seedlings under elevated CO_2 conditions may be attributed to the fact that, higher biomass allocation towards the height growth of the seedlings which has reduced the growth towards the lateral regions of the stem. Further, the nutrient deficit condition may also result in the production of thinner/leaner seedlings under elevated CO_2 condition. The reduction of stomatal density under elevated CO_2 condition could be attributed to the fact that, as the CO_2 level in the air surrounding the leaf increases, a lesser volume of air is necessary to obtain the requisite amount of CO_2 for the photosynthesis which implies that fewer stomata are sufficient to satisfy the need for gas exchange in the leaf. The present findings were also similar to the observations made by Ainsworth and Rogers (2007).

CONCLUSION

The results revealed that elevated CO_2 condition had significantly impacted the seed germination of *C. inophyllum.* The elevated CO_2 condition negatively influenced the germination and its parameters. But, after the germination, elevated CO_2 condition positively influenced the seedling height growth. The stomatal density of the seedlings was negatively affected by the elevated CO_2 condition. The supplementation of nutrients may increase the growth potential of the seedlings under elevated CO_2 conditions.

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