Growth and physiological potential of *Terminalia arjuna* under elevated CO\(_2\) levels in Open top chamber condition

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Abstract
*Terminalia arjuna* is native to India and occurs naturally along the banks of streams and rivers. The species is characterized to dry deciduous forests. The present study was carried out for the growth and physiological changes of *T. arjuna* in different elevated CO\(_2\) levels. Open top chambers were used to expose plants to ambient and elevated CO\(_2\) concentrations (400 and 800 ppm). The experiment was conducted in the month of March to August in 2019 for six months. The results showed that the growth parameters, i.e. plant height, collar diameter, the number of leaves, were found to be increased in elevated CO\(_2\) conditions. The percentage increase in physiological parameters like photosynthetic rate (28.82%), mesophyll efficiency (60% more in elevated CO\(_2\) condition), CO\(_2\) concentration (55% more in elevated CO\(_2\)), vapour pressure deficit (4.83 at 800 ppm) and water use efficiency (5.94 at ppm) increased. In contrast, transpiration rate (5.38 at 800 ppm and 10.11 ppm at ambient condition) and stomatal conductance (30% less in 800 ppm) decreased under elevated CO\(_2\) compared to ambient conditions. The study concluded that changing climatic conditions and significantly elevated CO\(_2\) in future may profoundly influence plant growth and the physiological response of *T. arjuna*.

Keywords: Adaptations, Biomass, Carbon partitioning, Medicinal plants, Photosynthesis

INTRODUCTION
Increasing atmospheric CO\(_2\) concentration is generally expected to enhance plant growth, allocation and chemical composition of alkaloids in medicinal plants. There are reports that an increase in atmospheric CO\(_2\) concentration will increase the potential production of C\(_3\) crops at higher latitudes (Taylor et al., 2018). Studies indicate that late in addition to shifting phenology, plant species have started to conform to the latest climate adjustments through alteration at the metabolic and physiological levels of the species (Buckley et al., 2013). The increasing level of CO\(_2\) already forced living organisms to adapt to ecosystems either through shift their habitat or life cycles and started to evolve new morphological and physiological traits (CBD 2014; Zari 2014; Tilman and Lehman 2001). As CO\(_2\) level increases in the environment, the impact of increased CO\(_2\) concentrations on the adaptation of agricultural species has been understood widely. This information is required for understanding the adaptive behaviour, ultimately biomass, and yield in future. Typically plants respond to increases in CO\(_2\) by an increased rate of photosynthesis and greater water use efficiency. Thus, the study was performed on *Terminalia arjuna* to understand changes in growth response and physiological response, like photosynthetic rate, transpiration, and stomatal conductance at optimum 600 and 800 ppm CO\(_2\) conditions.
MATERIALS AND METHODS

Experimental design
The Open Top Chamber (OTC) facility with automated controlled conditions of CO₂, temperature and relative Humidity is established at Central Nursery of Forest Research Institute, Dehradun, which was used to carry out this study. Three chambers were used to carry out the experiment under controlled CO₂, temperature, and relative humidity conditions. The sensors for measuring CO₂, temperature and relative humidity were placed inside the chamber with the support of an Iron rod. The portable temperature, humidity, and CO₂ sensors were placed in each chamber as a monitor device to obtain CO₂, temperature, and relative humidity data. The 100% CO₂ gas of commercial-grade was supplied to the chambers through CO₂ gas cylinder and maintained at set level (800 ppm) using manifold gas regulators, pressure pipelines, solenoid valves, sampler, pump, CO₂ analyzer, PC linked Program logic control (PLC) and Supervisory Control and Data Acquisition (SCADA). Three chambers were used to carry out the study. One chamber was kept as ambient (CO₂ enrichment) and the second chamber was maintained with elevated CO₂ (600 ppm) and the third chamber with elevated CO₂ (800 ppm) throughout the study period (Fig. 1). Three seedlings of T. arjuna of the same height were put in each chamber in polybags of size dash. Equal water treatment was provided in all chambers. The parameters related to growth dynamics such as plant height, collar diameter, number of leaves were considered. The photosynthetic rate, transpiration rate, stomatal conductance and water use efficiency, intercellular CO₂ concentration, mesophyll efficiency, carboxylation efficiency and vapour pressure deficit were observed as the physiological response of plants to elevated CO₂ concentration.

The study was carried out for 6 months, from March to August in 2019 for 6 months. The growth parameters of T. arjuna such as plant height (cm), collar diameter (mm) and the number of leaves were observed under different concentrations of CO₂. The photosynthesis rate (μmol CO₂ m⁻² s⁻¹), transpiration rate (mmol H₂O m⁻² s⁻¹), stomatal conductance (mol H₂O m⁻² s⁻¹), water use efficiency (A/gs), intercellular CO₂ concentration (Ci), mesophyll efficiency, carboxylation efficiency and vapour pressure deficit were observed as the physiological response of plants to elevated CO₂ concentration with the help of IRGA (InfraRed Gas Analyzer, LICOR USA). All the data was recorded in fully expended form between 09:30 am and 12:30 pm in clear sky condition.

RESULTS AND DISCUSSION

Growth parameters
The results related to growth studies in T. arjuna are represented in Fig. 2 and 3. The plant height was increased with increasing CO₂ concentration in comparison to ambient conditions. After 30 days, the average growth parameters of T. arjuna are shown in Fig. 2.
The plant height at ambient was 40.17 cm, whereas under elevated CO₂ (600 ppm), it was 42.83 cm and at 800 ppm, it was 46.50 cm. The collar diameter was increased with increasing CO₂ concentration in comparison to ambient air. After 30 days, the average collar diameter at ambient was 4.57 mm whereas under elevated CO₂ (600 ppm) it was 5.14 mm and at 800 ppm it was 5.58 mm (Fig. 2). The number of leaves increased with an increase in CO₂ concentration compared to ambient air. After 30 days, the average number of leaves was a maximum of 26 at 800 ppm followed by 20 at 600 ppm and 15 at ambient (Fig. 3).

The plant height was found to increase under elevated CO₂ compared to control. The plant's maximum height (47 cm) was attained at 800 ppm, while the minimum (40 cm) was ambient. As far as plant height and collar diameters were concerned under elevated CO₂ conditions, the plant produced more collar diameter and maximum (5.7 cm) under elevated CO₂ and minimum (4.0 cm) height and collar diameter was recorded in ambient CO₂. The number of leaves was recorded maximum under elevated CO₂ and minimum under ambient CO₂ conditions.

Different studies on rice have also shown that elevated CO₂ generally increased tiller number, photosynthesis, plant biomass and grain yield (Chakrabarti et al., 2012). The study of Thinh et al. (2017) and Saravanan and Karthi (2014) on Catharanthus roseus showed that plant height and number of leaves were significantly increased in elevated levels of CO₂ concentration. The results of these studies were quite similar to the present study on T. arjuna.

**Physiological traits**
The physiological traits such as photosynthetic rate and water use efficiency (WUE) increased. In comparison, transpiration and stomatal conductance decreased with an increase in the concentration of CO₂ (Fig. 4). The photosynthetic rate (Pn) was maximum (28.83) at 800 ppm and 25.20 at 600 ppm. The minimum (15.11) was recorded at ambient condition. The photosynthetic rate was 47% more in elevated CO₂ than ambient CO₂. These results were quite similar to the results of Sharma et al. (2018) and Nowak (2004), who reported that the increasing concentration of CO₂ influenced the mechanism of plant photosynthesis, metabolism and development in the growing concentration of CO₂ on plants Withania somnifera.

The effect of elevated CO₂ on tea (Camellia sinensis) plants was studied by Li et al. (2017) and similar results are reported in the case of photosynthetic rate, which was much higher (+87.9%) in case of short duration (24 days) of elevated CO₂ treatment in tea plants. In the present study, the rate of transpiration was maximum (10.117) at ambient condition and it was 46% more than elevated CO₂ which was 5.387. Thus, the transpiration rate was found to decrease under elevated CO₂ conditions.

WUE at ambient CO₂ was 1.49, whereas at elevated CO₂ (600 ppm), it was 5.94 and at 800 ppm of CO₂, it was 5.35. Thus 61% more stomatal conductance was observed in ambient as compared to elevated CO₂ condition (Fig. 5). It is reported that the increased photosynthetic process results in increasing plant height, stem diameter, the number of leaves and the number of branches under elevated CO₂ concentration in Pisum sativum (Kumari et al., 2016) and in C. roseus species (Saravanan and Karathi, 2014). In the present study, the maximum intercellular CO₂ concentration of 498.54 was observed under elevated CO₂ at 800 ppm, followed by 374.75 at 600 ppm. The minimum intercellular CO₂ concentration of 320.65 was recorded at ambient condition, which was 55% more intercellular CO₂ concentration under elevated CO₂ conditions than ambient conditions.

Carboxylation efficiency 0.06 at 600 ppm was significantly more and (0.04 at 800 ppm) as compared to control. This was also observed by Saravanan (2014) in Tectona grandis. The rate of mesophyll efficiency at ambient was 933.84, whereas at elevated CO₂ of 600 ppm.
Transpiration rate and mesophyll efficiency:
The maximum transpiration rate (10.38) was found in ambient CO\textsubscript{2} level and minimum (5.05) at elevated CO\textsubscript{2} (800ppm). 1.6% less transpiration rate was recorded in elevated CO\textsubscript{2} condition than ambient, 46% more transpiration rate was recorded in ambient CO\textsubscript{2} compared to elevated CO\textsubscript{2} and (Fig. 5). These results are reverse from photosynthetic rate and carboxylation efficiency of the plant (Fig. 4). Ping li et al. (2017) recorded a significant reduction in transpiration rate \((T_t)\) and stomatal conductance \((g_s)\). A large increase in water-use efficiency contributed to an increase in net photosynthetic rate \((P_n)\) under elevated \((CO_2)\) in Isatis indigo. Similar results were reported by (Singh et al., 2017) on roadside trees \((Lagerstroemia speciosa\) L) that exhibited reduced transpiration rate \((42.14 \%)\), decreased stomatal conductance \((66.85\%)\) and increased water use efficiency \((9.4\%).\)

Carboxylation efficiency
The results related to carboxylation efficiency are presented in Fig. 6. The efficiency was significantly maximum of 0.06 at 600 ppm, then 0.04 at 800 ppm, and then 0.04 at ambient. Furthermore, a tendency of higher value was observed for the intrinsic mesophyll efficiency (Fig.5) that was 20 \% more at elevated CO\textsubscript{2} (800ppm) as compared to optimum in present work, the carboxylation efficiency and mesophyll efficiency were found to increase in elevated CO\textsubscript{2}. These findings are quite similar to the earlier studies of Singh et al. (2018) and (Singh et al. 2016) for Ocimum tenuiflorum.

In the present study, 61\% more stomatal conductance was observed in ambient as compared to elevated CO\textsubscript{2} conditions. 72 \% more WUE was recorded under elevated CO\textsubscript{2} conditions of 800ppm. The decrease in stomatal conductance under elevated CO\textsubscript{2} conditions may limit the CO\textsubscript{2} fixation rate, promoting WUE. Similar results were reported by Sreeharsha et al. (2015), where CO\textsubscript{2} induced increase in WUE and photosynthetic rate significantly increased the growth (36.8\% more biomass } in Cajanus cajan (Pigeon pea). These results showed that these plants could perform well within a climate change context where water shortage periods, atmospheric temperature, and pores’ density allow the plants to breathe less.

As a consequence of this phenomenon, plants typically exhibited greater water-use efficiencies at higher levels of atmospheric CO\textsubscript{2}, which is a good sign of ecological adaptation for plants. In the present work, the stomatal conductance \((G/S)\) was reported maximum \((0.2652)\) at ambient, whereas at elevated CO\textsubscript{2} (600 ppm), it was 0.0803 at 800 ppm, it was a minimum of 0.1030 (Fig.4.0). Stomatal behaviour and development are two important factors to adapt CO\textsubscript{2} intake for photosynthesis and water release for transpiration to balance CO\textsubscript{2} and water exchange through the leaf epidermis in a changing environment (Lawson et al., 2014, Gao et
In the present study, the stomatal conductance showed a decreasing pattern with increasing CO₂ levels. The decrease at elevated CO₂ conditions could be attributed to the rapid closing of stomatal guard cells to the elevated CO₂ condition.

Conclusion

The present study concluded that the growth and physiological aspects of the medicinal plant T. arjuna responded positively to the elevated CO₂ condition. The highest growth rate was noticed under the elevated CO₂ conditions of 800ppm and 600 ppm. The physiological parameters like photosynthetic rate, WUE, and growth parameters like plant height and number of leaves were more under elevated CO₂ conditions than the ambient and control. Therefore, this research will be helpful in future climatic conditions when atmospheric CO₂ levels may be high and such tree species will sequester more CO₂ than others. Such trees will be more in demand to sequester more atmospheric CO₂ because of their great medicinal importance and may be used as a carbon sink in the coming time. The impact of elevated CO₂ on the reproductive phase of the plants also needs investigation.

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Conflict of interest

The authors declare that they have no conflict of interest.

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