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Original Research Paper

Growth and physiological response of mango (*Mangifera indica* L.) cv. Alphonso under elevated CO_2 conditions

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ABSTRACT

Atmospheric CO_2 concentration is expected to reach 460-560 ppm by the year 2050 with an increase of 3.2-4.0°C in temperature. Elevated CO_2 and temperature affect fruit crops to a greater extent by affecting flowering, yield and quality of fruits. In the current study, the effect of eCO_2 on mango cv. Alphonso was examined under open top chambers (OTC), with ambient CO_2 (380 ppm) and elevated CO_2 (550 ppm) levels, which were compared with the plants grown outside OTC under ambient conditions. The results revealed that the maximum number of vegetative shoot emergences was observed in OTC under both eCO_2 and aCO_2 conditions. The photosynthetic rate declined by 25% inside OTC due to increased air and leaf temperature compared to ambient plants placed outside the chambers. Significantly higher reproductive shoots emerged under aCO_2 conditions, whereas, no reproductive shoots were observed in aCO_2 under OTC, however, few reproductive shoots were observed under eCO_2 in OTC. The stomatal number was increased inside OTC chambers under $aCO_{2,}$ but the same was not observed under eCO_2 conditions. The other physiological parameters, such as specific leaf weight, chlorophyll content, relative water content, stem girth and total wax content were appeared to be better in eCO_2 conditions compared to aCO_2 inside OTC and ambient conditions outside OTC. The increase in stomatal number and complete repression of flowering inside OTC at aCO_2 was mainly due to higher temperatures compared to outside and this effect of temperature was reduced by eCO_2. The results of the study indicated that eCO_2 may improve growth rates, flowering and reduce water loss in mango plants.

Keywords: Alphonso, elevated $CO_{2,}$ flowering, OTC, RWC, stomatal density

INTRODUCTION

Carbon dioxide (CO_2) is the prominent greenhouse gas found on earth. Atmospheric CO_2 concentration is expected to reach 460-560 ppm by 2050, with a raise in the temperature range of 2.6-4.8 °C (IPCC, 2013). Leaf photosynthetic rate is greatly influenced by temperature, light, and CO_2 concentrations. Among them, elevated CO_2 has a positive effect on photosynthesis, but the rise in temperature can nullify this effect of elevated CO_2. Increased temperature will also lead to more rapid growth and development of plants, and it may delay the growth cessation of perennial plants at the beginning of the winter. Whiley et al. (1989) also reported that the mean leaf mass of trees grown under 30/25°C (day/night temperature) was 300% bigger than that of trees growing at 20/15°C. Whereas, Dambreville et al. (2013) did not observed significant effect of temperature on leaf mass in the mean daily temperature range of 20-28°C. There are no published works available on the effect of elevated CO_2 and temperature on mango cv. Alphonso; therefore, the present study was undertaken to understand the growth and physiological response of mango plants.

MATERIALS AND METHODS

The study was carried out at ICAR-Indian Institute of Horticultural Research, Bengaluru (13°582 N latitude, 78° E longitude, and 890 m above mean sea level), India during 2017 to 2019. The mango cv. Alphonso, one of the highly commercially important cultivars considered for current study and a total of five uniformly grown plants were used for each treatment. The treatment consists of elevated CO_2 (550 ppm), ambient CO_2 conditions (380 ppm) inside the open top chamber (OTC) and ambient conditions outside the OTC. The observations such as vegetative and reproductive flushes, photosynthetic rate, specific leaf weight (SLW), chlorophyll content, relative water content (RWC), stem girth, total wax and stomatal density (SD) were monitored continuously.

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Structural feature of OTC

To evaluate the performance of horticultural crops under elevated CO_2 concentrations, OTC was used. The whole chamber is fitted with transparent, polyvinylchloride (PVC) sheet (150-micron gauge) measuring 3 m x 3 m x 2.4 m (l x b x h) to provide the maximum amount of sunlight to the plants. The top portion of the chamber is left open to avoid excessive build-up of temperature and CO_2 is pumped into these chambers continuously to raise CO_2 concentration, which was monitored using Delta T CO_2 monitoring systems. The temperature and humidity sensors (Kestrel Drop D_2, NK Kestrel, USA) were installed outside and inside the OTC to record temperature and humidity at a 5-minute interval and stored them in a data logger.

Once the plants were shifted to OTC, vegetative and reproductive flushes were recorded continuously. The dates of the emergence of new flushes were recorded as and when they emerged. Gas exchange characteristics like the net photosynthetic rate were measured from the fully matured, recently emerged leaf between 09.30 h and 11.00 h using portable photosynthesis system, LICOR LI-6400XT, Lincon, USA. The PAR of 1000 µmol m^{-2} s^{-1} using the light source and the ambient CO_2 concentration of 400 µ mol mol^{-1} were maintained during the measurements. Specific leaf weight (SLW) is a measure of leaf weight per unit leaf area. The dry weight of a known area of leaves was recorded and the SLW was calculated by dividing the total dry weight by its area and expressed as g cm^{-2} as suggested by Pearce et al. (1969). Stem girth is a measure of the growth rates of a plant. For each treatment, five plants were used to record the girth of primary branches at a fixed position on the stem. Relative water content was measured by following the Gao (2006) method with slight modifications and the results were expressed in terms of percentage reduction. Epicuticular wax was estimated using the chloroform method as suggested by Ebercon et al. (1977) with a slight modification. The concentration of leaf wax was calculated from a standard curve prepared using Nonadecane and expressed as µg/cm^2. Total chlorophyll in mango cv. Alphonso leaf was measured by following the traditional method of Hiscox & Israelstam (1979) with slight modifications. Stomatal density was estimated by the leaf impression method, where nail polish imprints obtained directly from the abaxial side of mature leaves were observed under a light microscope (Olympus BX61). The results were expressed as the number of stomata per mm^2 of leaf area.

Statistical analysis

Data were subjected to the analysis of variance using ANOVA and means were compared with the critical difference at $P<0.05$.

RESULTS AND DISCUSSION

Mango cv. Alphonso plants were grown under eCO_2 (550 ppm) and aCO_2 (380 ppm) conditions inside OTC and compared with plants grown under ambient condition outside OTC (Fig. 1). Daily mean maximum and minimum temperatures under OTC with eCO_2 conditions (550 ppm) were increased by 3-4°C. The mean maximum and minimum temperatures during the experimental period are presented in Table 1 and it was also found to increase from 2017-18 to 2018-19.

Fig. 1 : Control (A) and elevated CO_2 (B) mango plants during 'on' season

Vegetative and reproductive flushes were recorded from 2017 to 2019 (Fig. 2a). Significantly higher numbers of reproductive shoots emerged under

Table 1 : Minimum and maximum temperature and relative humidity data during experimental period

Year	Elevated CO_2		Ambient CO_2		Ambient	
	Max (°C)	Min (°C)	Max (°C)	Min (°C)	Max (°C)	Min (°C)
2017-18	47.83	13.01	48.67	12.42	34.16	12.28
2018-19	48.03	13.92	49.18	13.01	35.09	13.02

ambient conditions outside OTC, and no reproductive shoot was observed in aCO_2 under OTC; however, a few reproductive shoots were observed under eCO_2 in OTC (Table 2). Biomass improvement under eCO_2 conditions has also been reported in many previous studies (Van der Kooi et al., 2016). The maximum number of vegetative shoot emergences was observed in both eCO_2 and aCO_2 conditions under OTC (Fig. 2b). High temperatures under OTC conditions completely repress reproductive growth; however, CO_2 may promote reproductive growth if temperature is normal, as observed by the limited number of reproductive shoots under eCO_2 conditions inside OTC.

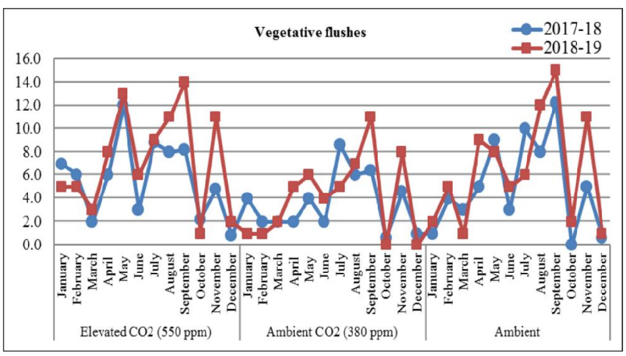


Fig. 2a : Mean number of vegetative shoots emerged under elevated CO_2, ambient CO_2 inside OTC and ambient outside OTC

Table 2 : Total number of vegetative and reproductive shoots under elevated CO_2, ambient CO_2 inside OTC and ambient outside OTC

Treatment	Vegetative shoots	Reproductive shoots
Elevated CO_2 (550 ppm)	28	5
Ambient CO_2 (380 ppm)	42	0
Ambient	19	47

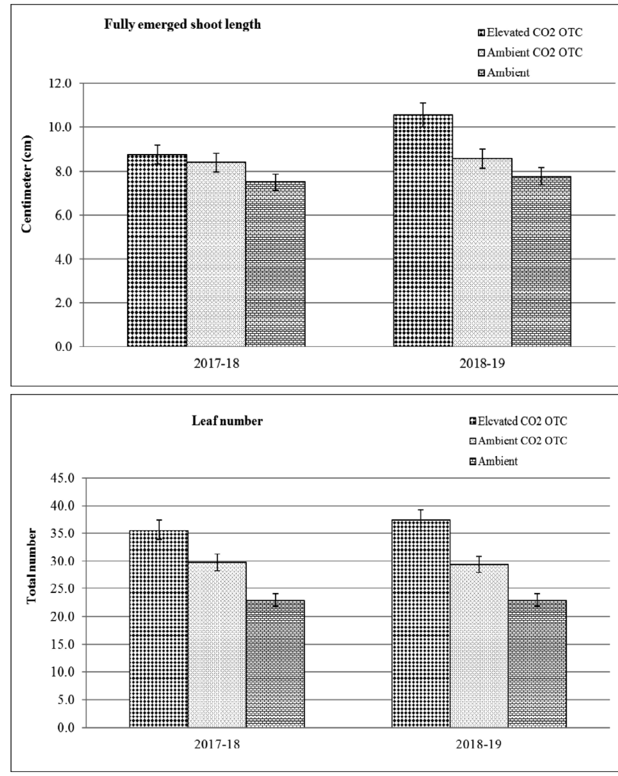


Fig. 2b : Shoot length and leaf number emerged under elevated CO_2, ambient CO_2 inside OTC and ambient outside OTC

Gas exchange characteristics revealed that the OTC can increase air and leaf temperatures. Plants under eCO_2 conditions showed the highest photosynthetic rates and the lowest stomata conductance and transpiration (Table 3). Elevated CO_2 causes increased photosynthesis in plants, which leads to greater production of biomass (Thompson et al., 2017). Higher temperatures decrease photosynthesis but increase transpiration and stomatal conductance, leading to a shorter growth period and faster development (Lobell & Gourdji, 2012).

Table 3 : Gas Exchange parameters under elevated CO_2, ambient CO_2 inside OTC and ambient outside OTC

CO_2 concentration (ppm)	Elevated CO_2 (550 ppm)	Ambient CO_2 (380 ppm)	Ambient
400 ppm	7.071	7.269	7.051
600 ppm	9.239	12.732	11.803
800 ppm	11.424	15.195	14.437
Mean	9.244	11.732	11.097
CD for CO_2 concentration		1.751	
CD for treatments		2.145	
CD for (C X T)		N/A	

Specific leaf weight increased under eCO_2 compared to aCO_2 inside OTC, and ambient outside OTC and this is probably due to the higher photosynthetic rate under eCO_2 conditions, which causes an increase in the SLW of leaves (Fig. 3). Increased thickness is one of the common responses to eCO_2, may be due to an increased non-structural carbohydrate and more cell layers; sometimes both can contribute to the thickness (Ferris et al., 2001).

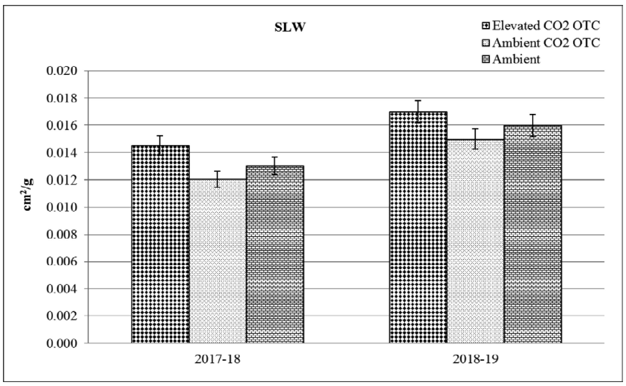


Fig. 3 : Specific leaf weight (SLW) under elevated CO_2, ambient CO_2 inside OTC and ambient outside OTC

Stem girth is the measurement of the growth rate of trees, and in the present studies, it did not show a much significant difference between the treatments such as eCO_2 (3.7 cm), aCO_2 (3.4 cm), and ambient (2.8 cm) (Fig. 4). Similar results were also reported by David & Christopher (2011) and Attipalli et al. (2010), where growth parameters, *viz.*, height, diameter, and volume were increased by eCO_2 and were not significantly different from the ambient control under eCO_2.

Relative water content was in the range of 80-90% with higher values in eCO_2, followed by aCO_2 inside OTC, and lowest was in the ambient outside OTC (Fig. 5). This might be due to the lower transpiration rate of the leaves under eCO_2 conditions in OTC. The effects of eCO_2 concentration on the leaf water loss dynamic responses of in vitro-produced walnut leaves during ex vitro desiccation were investigated. Higher CO_2 concentration resulted in a lower transpiration rate and a higher RWC during *ex vitro* desiccation. This improvement was due to decreased stomatal aperture during the first phase of water loss (Vahdati et al., 2017).

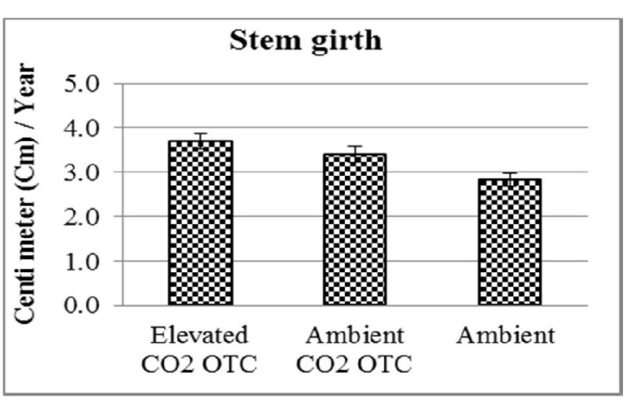


Fig. 4 : Stem girth in plants grown under elevated CO_2, ambient CO_2 inside OTC and ambient outside OTC

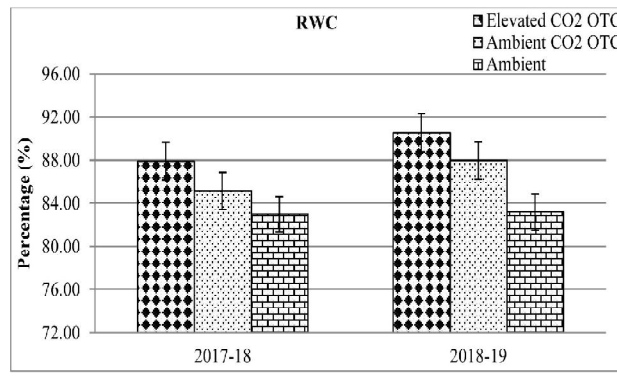


Fig. 5 : Relative water content (RWC) content in plants grown under elevated CO_2, ambient CO_2 inside OTC ambient outside OTC

Epicuticular wax content was higher in eCO_2 conditions compared to aCO_2 inside OTC, and ambient outside OTC which might be restricting the transpiration rate during the night leading to better RWC (Fig. 6). Shepherd & Griffiths (2006) reported that cuticular wax content was significantly increased under eCO_2 to provide protection against UV-B.

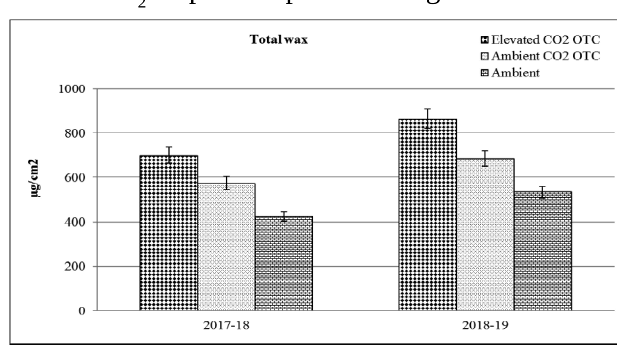


Fig. 6 : Epicuticular wax in plants grown under elevated CO_2, ambient CO_2 inside OTC and ambient outside OTC

Total chlorophyll was observed to be high in eCO_2 compared to aCO_2 inside OTC and ambient outside OTC (Fig. 7). Earlier reports on total chlorophyll content were reported to decrease under eCO_2 conditions (Byeon et al., 2021).

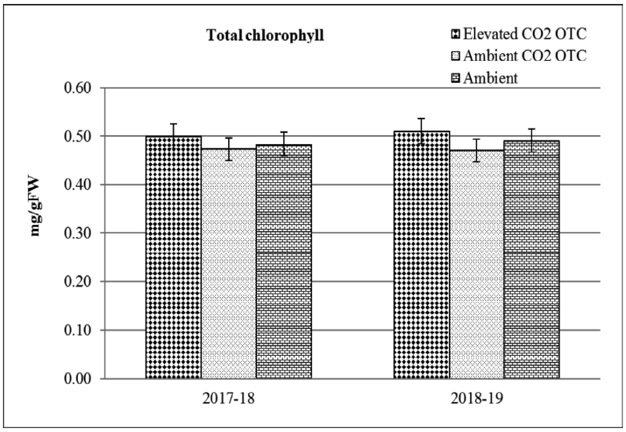


Fig. 7 : Total chlorophyll content in plants grown under elevated CO_2, ambient CO_2 inside OTC and ambient outside OTC

Stomatal density results are in line with the previous study, which revealed that higher temperature in aCO_2 increased the stomatal number (Fig. 8a), however, eCO_2 was found to nullify this effect (Fig. 8b). Generally, eCO_2 causes a reduction in stomatal density (Teng et al., 2009), stomatal conductance (Ainsworth & Rogers, 2007), leaf transpiration (Katul et al., 2010), and evapotranspiration (Leakey et al., 2009).

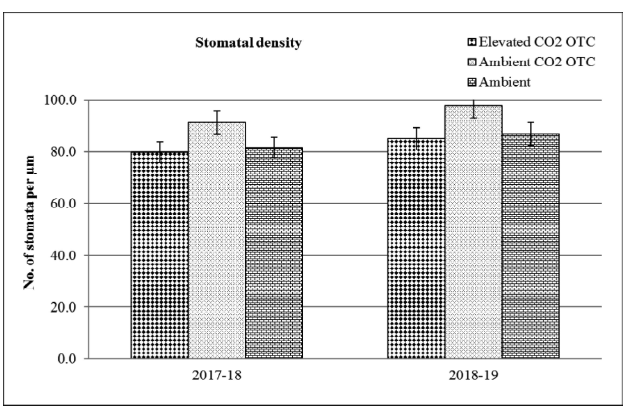
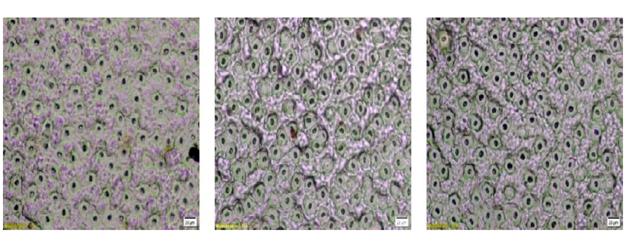


Fig 8a : Stomatal number in plants grown under elevated CO_2, ambient CO_2 inside OTC and ambient outside OTC



Elevated CO_2 Ambient CO_2 Ambient

Fig 8b : Microscope images of stomata under elevated CO_2, ambient CO_2 inside OTC and ambient outside OTC

CONCLUSION

Mango plants responded to eCO_2 conditions by decreasing the stomatal number, increased SLW, and increasing the growth rate. Elevated temperature completely inhibits the reproductive growth and promotes vegetative flushes, and increases the stomatal number. However, eCO_2 may increase reproductive shoot emergence and leaf wax content and reduce the transpiration rate.

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