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Impact of elevated CO_2 and temperature on rooting of grape cuttings

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Abstract
An experiment was carried out to study the impact of eCO_2 (550ppm), eT (+3ºC) and their interaction (eCO_2+eT) on rooting behaviour of cuttings of three grape varieties (Thompson Seedless, Bangalore Blue, and Dogridge) in FATE and OTC facilities. Observations were recorded at 50 and 80 days after planting (DAP) and root growth data was recorded and analysed using WinRHIZO root scanner and its software. Analysis revealed that, among the selected grape varieties, Thompson Seedless cuttings has shown highest number of roots, root volume and dry biomass under eCO_2 and eCO_2+ eT conditions, while total root length and root length density were highest with Bangalore Blue. Under eT condition, Bangalore Blue showed maximum number of roots, total root length and root length density, while root volume and dry biomass was highest in Thompson Seedless. The values of root parameters under all conditions and their response to eCO_2 was lowest in Dogridge. Though eT condition reduced all the root parameters, their performance improved under eCO_2+ eT indicating the presence of higher concentration of CO_2 reduced the ill effects of high temperature. Overall, eCO_2 and eCO_2+eT conditions improved root parameters of grape varieties, while eT reduced them as compared to their performance under ambient condition and varietal variation is significant.

Keywords: eCO_2, root growth, stem cuttings, varieties, WinRHIZO

Introduction
Globally, climate change is an important issue which is showing a significant impact on agricultural production and productivity. The climatic parameters are showing a noteworthy change in their patterns like rise in atmospheric CO_2, temperature, changes in precipitation patterns, UV radiation and higher incidence of extreme weather events such as floods, heat waves etc., which are emerging as major threats for agricultural production. The climate change is mainly as a result of emission of greenhouse gases such as CO_2, CH_4, N_2O etc., which will cause global warming (IPCC 2007) [8] and will affect all levels of life from species to ecosystem. An atmospheric concentration of CO_2 is steadily increasing year by year, and at this rate of increase, the concentration of CO_2 is projected to reach between 700 and 1000 ppm by 2100 (IPCC 2014) [9]. Rising concentrations of carbon dioxide will potentially increase global average surface temperatures by 1.4-5.8ºC. Therefore, it is important to quantify the interactive effects of increasing CO_2 and temperature on crop production.

Grape (*Vitis vinifera* L.) is sensitive to different environmental factors, including temperature, water availability and CO_2. Like other C3 plants, grapevine photosynthesis is CO_2 limited (Mullins *et al.*, 1992) [12] and any increase of atmospheric CO_2 concentration could enhance accumulation of vegetative biomass and increase fruit yield (Bowes 1993; Rogers *et al.*, 1994) [2, 18]. Impact analyses of plant responses to elevated atmospheric CO_2 have focused largely on aboveground processes and very limited studies were on root growth and development especially with fruit crops. It is necessary to understand the response of roots towards elevated CO_2 condition as roots are interface between the lithosphere and biosphere. Root health in crop plants will play a major role in providing sustainable productivity as it enables to cope with changed climatic conditions. As grape is propagated by hardwood cuttings, better root production helps in better establishment in field. Keeping this in view the present research work was carried out with the objective to study the effect of elevated carbon dioxide and temperature on rooting of grape cuttings as well as variability in response of three popular grape cultivars.

Materials and Methods

Plant materials

Hard wood stem cuttings of 25-30 cm length having 5-6 nodes of three popular grape varieties i.e. Thompson Seedless, Bangalore Blue, Dogridge were collected from the winter pruning of grape plants. Hard wood stem cuttings were treated with standard 1500 ppm IBA (Lab grade) solution and planted in poly bags. The poly bags are of 23×12×12 cm and filled with potting mixture of FYM+ Red Soil in 1:1 proportion and 15 number of cuttings each variety were exposed to ambient control, eCO_2, eT and eCO_2+eT conditions. The planted cuttings were kept under specially designed Open Top Chambers (OTC) and Free Air Temperature Enrichment (FATE) facilities in Rabi 2017 at ICAR-CRIDA. The observations were recorded on three cuttings of each variety from all the conditions (ambient control, eCO_2, eT and eCO_2+eT) at 50 and 80 days after planting (DAP).

Treatments and Experimental conditions

To study the rooting pattern, the cuttings planted in polybags were exposed to elevated carbon dioxide of 550ppm in OTCs and elevated temperature (ambient+3 °C), elevated carbon dioxide (550ppm) + elevated temperature in FATE facility.

In OTC (3 x 3 x 3 m) the desired levels of CO_2 (550ppm) concentration was maintained by continuous injection of CO_2 into the plenum of OTCs where it was mixed with air from air compressor before entering chamber and the set level of CO_2 concentration was maintained with the help of solenoid valves, rotometers, programme Logic Control (PLC) and Supervisory Control and Data Acquisition (SCADA) software.

Free Air Temperature Elevation (FATE) facility consisting of six rings fitted with 24 arrays of 2000 W capacity ceramic infrared heaters above the canopy to maintain elevated crop canopy temperature (eT) of ambient +3 °C. Among these, three rings were also provided with CO_2 release system at 0.3 m height from base of the ring to study the interactive effects of temperature and CO_2 (eT + eCO_2). The polyurethane (PU) tubing with perforations releases the CO_2 within the ring to maintain the elevated concentration of 550 ppm. The CO_2 in turn regulated by SCADA based control system linked with CO_2 analyser, wind direction and wind speed. The ambient control was maintained without any enhancement of CO_2 and temperature.

WinRHIZO is an image analysis system specially designed for root measurement in different forms. The cuttings were harvested at 50 and 80 DAP and the roots were thoroughly washed to remove all soil particles. Scanning and image analysis was carried out using WinRHIZO root scanner (LA-1600) for root characteristics and the root morphology and architecture measurements such as total root length, root diameter, root length density and root volume were done by WinRHIZO program.

Results and Discussion

1. Root number (No.of roots P^{-1})

At 50 DAP, Thompson Seedless (10.2±0.182) and Dogridge (6.3±0.54) showed maximum number of roots under eCO_2+eT, whereas in Bangalore Blue the maximum number of roots were found under eT (10.3±0.23) condition. While at 80 DAP, all the three varieties have shown highest number of roots under eCO_2condition. Among the three grape varieties, Thompson Seedless has shown higher number of roots under eCO_2 and eCO_2 + eT, while Bangalore Blue under eT and Dogridge under ambient conditions (Table 1a).

Root growth of most of the crops is enhanced under elevated atmospheric CO_2 (Rogers *et al.*, 1994; Pritchard and Amthor, 2005) [2, 15], often to a greater extent than leaves, stem, and reproductive structures (Norby *et al.*, 2001 and Kimball *et al.*, 2002) [14, 10]. According to Madhu and Jerry (2015) [11], high CO_2 might have inhibited the growth at early stage of crop but due to adoptive mechanism to high CO_2 plants were recovered and responded physiologically at later stage. This is in accordance with Dogridge cuttings under eCO_2 at 50 DAP. The eCO_2 increases the carbon flow to the rhizosphere by increasing photosynthetic activity which in turn improves root production in plants (Rajkumar *et al.*, 2013). More roots at all depths of the soil profile have been observed in sorghum when exposed to elevated CO_2 (Chaudhuri *et al.*, 1986) [3].

2. Total Root length (cm)

At 50 DAP, Thompson Seedless (309.0±30.4 cm) and Bangalore Blue (365.4±24.5 cm) have shown higher root length under eCO_2+eT while Dogridge has shown higher root length under eT (165.0±9.2 cm). At 80 DAP Thompson Seedless (336.6±27.8 cm) and Dogridge (227.2±12.1 cm) has shown highest root length under eCO_2+eT, while Bangalore Blue under eCO_2 (420.5±63.1 cm) (Table 1a). This clearly indicating that individual condition of eCO_2 promoted higher root length while eT showed very less impact especially in Thompson Seedless and Bangalore Blue and the combined conditions of eCO_2 and *et al.*so improved the root length though with lesser magnitude, indicating the impact of eCO_2 even at higher temperature on this trait. Though Dogridge registered no response for root length under eCO_2 recorded better response under eT and eCO_2+eT revealing its tolerance to higher temperature.

Rogers *et al.* (1992) [2] demonstrated that increased CO_2 level resulted in enhanced root growth in soybean and Wang *et al.* (2013) [21] recorded increased root length in tomato at elevated CO_2 than ambient CO_2 condition. Soybean produces more biomass and longer roots under eCO_2 (Del Castillo *et al.*, 1989) [6]. Davis and Potter (1983) [5] observed that elevated CO_2 increased root length and dry weight of several ornamentals.

3. Root Length Density (cm cm^{-3}):

Thompson Seedless has shown higher Root Length Density (RLD) under eCO_2+eT at 50 DAP (0.09±0.01) and 80 DAP (0.10±0.01), while Bangalore Blue has shown higher RLD under eCO_2+eT (0.11±0.01) at 50 DAP, and under eCO_2 (0.13±0.02) at 80 DAP. However, Dogridge has shown higher RLD under eT (0.05±0.00) at 50 DAP, and under eCO_2+eT (0.07±0.00) at 80 DAP (Table 1a).

Among the three varieties, Bangalore Blue has shown higher RLD than Thompson Seedless and Dogridge under all the treatments. This may be because of the difference in root system of Bangalore Blue like having more root length, producing a greater number of roots, and production of number secondary roots and root hairs. From these results it can be concluded that RLD is higher when cuttings exposed to eCO_2 alone or in combination with high temperature (eCO_2+eT).

4. Root volume (ml pl^{-1})

At 50 DAP, Thompson Seedless has shown higher root volume under eCO_2+eT (8.0 ml/pl). Though the root volume of Thompson Seedless was less under eCO_2 at 50 DAP (2.0 ml/pl), however by 80 DAP it was highest (21.0 ml/pl). In Bangalore Blue the root volume was highest under eCO_2 and similar (8.5±0.37) at both 50 and 80 DAP. While in Dogridge highest root volume was under eCO_2+eT at 50 (3.0±0.0) and 80 (4.0±0.0) DAP. Under eCO_2 and control conditions there was no significant difference in root volume of Bangalore Blue at 50 and 80 DAP, whereas a significant difference plants under eT and eCO_2+eT shown at 50 and 80 DAP. At final sampling Thompson Seedless has shown higher root volume under all the three treatments (Table 1a) as compared with ambient control.

5. Dry weight of roots (g pl^{-1})

Maximum root biomass of Thompson Seedless (4.4±0.27) and Bangalore Blue (1.7±0.07) has shown under eCO_2, while Dogridge has shown the highest root biomass under eCO_2+eT (0.8±0.03) at 50 DAP. At 80 DAP Thompson Seedless (3.6±0.12) and Dogridge (1.2±0.05) roots have shown maximum dry weight under eCO_2, however Bangalore Blue has shown maximum root biomass under eCO_2+eT (2.3±0.10). Among the selected three different varieties, maximum biomass was accumulated by Thompson Seedless and lowest by Dogridge under all the four treatments at both 50 and 80 DAP while plants under ambient condition recorded lowest biomass in all the three varieties (Table 1a).

At final sampling, all the treatments have shown an increase of root dry mass over control. Under eCO_2, Thompson Seedless has shown maximum root dry weight, while Dogridge under eT and eCO_2+eT.

The dry matter accumulation is resultant effect of enhanced plant growth characters and greater photosynthetic accumulation. The amount of dry matter produced depends on the better photosynthesis obtained by large and efficient assimilating area, adequate supply of solar radiation, carbon dioxide, favourable environmental condition and efficient utilization of nutrients (Reddy *et al.*, 1994) [17]. Many studies have observed a greater root biomass under elevated eCO_2 (Chaudhuri *et al.*, 1990, Acock *et al.*, 1990; Weigel *et al.*, 1994) [14, 1, 22]. Cotton produces heavier roots (Reddy *et al.*, 1994) [17] under eCO_2. In 2013-15, on experiments with *Valeriana jatamansi*, Munish Kaundal and Rakesh Kumar (2020) [13] found Elevated CO_2 significantly enhanced root biomass of *Valeriana jatamansi*. The results are in line with earlier reports on different plant species where Goudriaan and Ruiter (1983) [7] found that doubling of eCO_2 had the largest effect on dry matter production provided nutrient supply is not limiting.

6. Average root diameter (mm)

At 50 DAP, average root diameter of Thompson Seedless was 1.2 mm at both eCO_2 and eT, while under eCO_2+eT the diameter was 1.1 mm, however under control the average diameter was 2.1 mm. In Bangalore Blue the average diameter was 0.9 mm under eCO_2, 1.4 mm under eT, 0.6 mm under eCO_2+eT and 1.7 mm under control treated plants. In Dogridge the average diameter was 0.9 mm under eT, 1.4 mm under eCO_2+eT and 1.5 mm under control (Table 1a)

At 80 DAP, the average root diameter of Thompson Seedless was 0.8 mm under eCO_2, 1.5 mm under eT, 0.9 mm under eCO_2+eT and 1.2 mm under control. In Bangalore Blue the average diameter was 0.7 mm under eCO_2, 1.1 mm under eT, 0.8 mm under eCO_2+eT and 1.0 mm under control treated plants. In Dogridge the average diameter was 0.7 mm under eCO_2, 0.9 mm under eT, 1.4 mm under eCO_2+eT and 1.5 mm under control.

It is known that lower the diameter of root, higher will be the water absorption. Thompson Seedless and Bangalore Blue has shown lowest root diameter (i.e higher number of fine roots) under eCO_2+eT at 50 DAP and under eCO_2 at 80 DAP Here we can conclude that the diameter of the root was lowest at high eCO_2 (eCO_2) alone or in the presence of high temperature (eCO_2+eT). In case of Dogridge the diameter was low under eT at first sampling, whereas at 80 DAP the diameter of the roots became lower under eCO_2 and eCO_2+eT. This may be due to initiation of fine roots under eCO_2 during early stage of growth for Dogridge. The root diameter tend to be lowered from 50 to 80 DAP in all varieties at all treatments, the diameter of roots were highest under eT than eCO_2 and eCO_2 +eT this indicates that there would be involvement between eCO_2 and formation of fine roots in plants that were grown under eCO_2.

Table 1a: Mean (± SE) performance of root parameters of grape varieties under eCO_2, eT, and interaction (eCO_2+eT) at 50 and 80 DAP:

Treatments	Varieties	Number of roots/pl		Total root length (cm)		Root length density (cm/cm^3)		Root volume (ml/pl)		Dry weight (g/pl)		Average root diameter (mm)	
		50 DAP	80 DAP	50 DAP	80 DAP	50 DAP	80 DAP	50 DAP	80 DAP	50 DAP	80 DAP	50 DAP	80 DAP
eCO_2	TS	6.5±0.47	12.7±0.365	179.8±32.8	287.1±29.4	0.05±0.0	0.09±0.0	2.0±0.0	21.0±1.35	4.4±0.27	3.6±0.12	1.2±0.1	0.8±0.0
	BB	5.7±0.365	12.2±0.522	297.3±24.6	420.5±63.1	0.09±0.0	0.13±0.0	8.5±0.37	8.5±0.37	1.7±0.07	1.8±0.16	0.89±0.0	0.71±0.1
	DR	0.0±0.0	8.2±0.34	0±0	179.2±16.5	0.0±0.0	0.05±0.0	0.0±0.0	3.3±0.46	0.0±0.0	1.2±0.05	0±0.0	0.67±0.1
eT	TS	10±0.399	10.2±0.52	195.6±26.2	198.2±23.0	0.06±0.0	0.06±0.0	7.5±0.4	9.5±0.37	1.7±0.17	2.2±0.07	1.2±0.2	1.5±0.1
	BB	10.3±0.231	10.7±541	179.1±12.4	278.3±19.3	0.05±0.0	0.08±0.0	2.2±0.18	6.8±0.18	0.4±0.02	1.5±0.21	1.41±0.1	1.13±0.1
	DR	3.8±0.18	4.7±0.23	165.0±9.2	208.4±11.2	0.05±0.0	0.06±0.0	2.0±0.0	2.3±0.36	0.4±0.02	1.1±0.02	0.88±0.0	0.87±0.1
eCO_2+eT	TS	10.2±0.182	10.8±0.66	309.0±30.4	336.6±27.8	0.09±0.0	0.10±0.0	8.0±0.0	9.3±0.46	2.7±0.20	2.8±0.07	1.1±0.1	0.8±0.0
	BB	7.2±0.439	8.3±0.729	365.4±24.5	380.0±34.2	0.11±0.0	0.11±0.0	4.3±0.23	7.7±0.23	2.0±0.13	2.3±0.10	0.62±0.1	0.76±0.0
	DR	6.3±0.54	6.3±0.23	120.4±9.9	227.2±12.1	0.04±0.0	0.07±0.0	3.0±0.0	4.0±0.0	0.8±0.03	2.1±0.04	1.36±0.2	0.72±0.1
Control	TS	3.2±0.34	11.3±0.365	59.9±2.1	180.8±2.7	0.02±0.0	0.05±0.0	5.7±0.2	6.2±0.18	1.0±0.09	1.4±0.07	2.0±0.1	1.2±0.1
	BB	4.7±0.365	7.8±0.182	104.2±3.4	275.1±21.5	0.03±0.0	0.08±0.01	3.0±0.0	3.5±0.37	0.4±0.05	1.1±0.03	1.71±0.2	1.0±0.0
	DR	5.5±1.05	5.7±0.23	123.8±19.5	142.5±2.9	0.04±0.0	0.04±0.0	2.0±0.0	2.8±0.1	0.4±0.04	0.5±0.02	1.54±0.2	1.15±0.1

Table 1b: ANOVA for root parameters of grape varieties

Factors	Number of roots		Total root length		Root length Density		Root volume		Dry weight		Average root diameter	
	50 DAP	80 DAP	50 DAP	80 DAP	50 DAP	80 DAP	50 DAP	80 DAP	50 DAP	80 DAP	50 DAP	80 DAP
V	89.52**	160.87**	110296.17**	134849.75**	0.010**	0.012**	102.264**	424.625**	22.502**	10.030**	1.285**	0.510**
T	84.86**	30.014**	87711.952**	53503.306**	0.008**	0.005**	10.162**	144.981**	12.516**	6.903**	3.603**	0.989**
V*T	33.61**	7.236**	43618.615**	9780.032*	0.004**	0.001*	45.579**	70.829**	4.547**	1.046**	0.940**	0.116**
LSD (V)	0.816	0.749	33.979	44.315	0.010	0.013	0.335	0.867	0.196	0.169	0.227	0.140
LSD (T)	0.942	0.865	39.236	51.171	0.012	0.015	0.386	1.001	0.226	0.195	0.262	0.161
LSD (V*T)	1.632	1.498	67.959	66.619	0.020	0.020	0.669	1.733	0.391	0.338	0.453	0.279
CV (%)	17.34	10.75	25.28	22.23	25.24	22.23	10.85	15.93	17.67	12.28	25.15	19.18

V-Varieties; T-Treatments;**,*significance at $p \leq 0.01$, 0.05 respectively; NS-Non significant

Conclusion

The eT condition reduced all the root parameters, their performance improved under eCO_2+ eT indicating the presence of higher concentration of eCO_2 reduced the ill effects of high temperature. Overall, eCO_2 and eCO_2+eT conditions improved root parameters of grape varieties, while eT reduced them as compared to their performance under ambient condition and varietal variation happen to be significant. The amount of dry matter produced depended upon the better photosynthesis obtained by large and efficient assimilating area, adequate supply of solar radiation, carbon dioxide, favourable environmental condition and efficient utilization of nutrients.

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