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Short communication

Effect of elevated CO_2 levels in Kinnow mandarin and Kagzi Kalan lemon under controlled environment conditions

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ABSTRACT

With the countinious increment in global industrialization, productivity of horticultural crops is expected to be affected drastically by changing climate especially due to increasing of atmospheric carbon dioxide concentration. A study were carried out on one-year-old budded plants of Kinnow (*Citrus nobilis* × *Citrus deliciosa*) and Kagzi Kalan (*Citrus limon*) to assess the physiological and biochemical changes induced under elevated CO_2 concentration (450 and 550 ppm). The per cent increase in plant height (Kinnow - 17.06 & 6.80, Kagzi Kalan- 33.38 & 25.64) and leaf number (Kinnow - 14.20 & 22.92, Kagzi Kalan- 33.94 and 19.81) were significantly higher at 450 and 550 ppm, respectively over control. Initially, the photosynthetic rate was significantly increased over control (13.9 and 12.1 µmol CO_2 $m^{-2}s^{-1}$ at 550 ppm in Kinnow and Kagzi Kalan, respectively), with increasing CO_2 level regardless of genotype. The total chlorophyll content in both the species (3.15 & 2.32 mg/gFW), activity of catalase (1.32 and 1.44 (µ moles H_2O_2 hydrolyzed min^{-1} mg^{-1} protein), proline concentration (153.80 and 252.83 µg/g FW) and peroxidase (520.12 and 669.50 A_{436} unit min^{-1} µg g^{-1} FLW) were significantly higher in Kinnow leaved compared to Kagzi Kalan at both the CO_2 levels. Hence, Kinnow showed more tolerance as compared to Kagzi Kalan lemon for increasing CO_2 challenge.

Key words: Elevated carbon dioxide, citrus, photosynthesis, global climate change.

The earth's atmospheric carbon dioxide concentration has increased from a mean concentration of approximately 280 parts per million (ppm) since the start of the Industrial Revolution to about 380 ppm at present. Recent estimates of atmospheric CO_2 levels in ice cores have shown that global CO_2 has increased by about 60 µmol mol^{-1} over the past 200 years (Woodward, 12). There is strong evidence that plants have already responded to the 25% increase in atmospheric CO_2 concentration since the onset of the Industrial Revolution (Dippery *et al.*, 3; Vu, Joseph, 10). Approximately 95% of terrestrial plant species fix atmospheric CO_2 by the C3 photosynthetic pathway, while 1% by the C4 and 4% by CAM pathway (Bowes, 2). Current concentration of atmospheric CO_2 restricts the photosynthetic performance, growth and yield of many crop plants, including citrus. Since the predicted increase in atmospheric CO_2 concentration may affect biological processes, it is important to continue studying the direct effects of increasing CO_2 at levels ranging from the molecular to the global (Ward and Strain, 11).

The pot experiment was conducted during 2011-2012 on one-year-old budded plants of Kinnow (*Citrus nobilis* × *Citrus deliciosa*) and Kagzi Kalan (*Citrus limon* Burm.) grown in a polyhouse under the open field conditions before transferring them to National Phytotron Facility (day temp., 25-32°C; night temp., 15-18°C; relative humidity 60-90%). These plants were grown in plastic pots (12' size) containing 7 kg of potting mixture of garden soil and well-rotten farmyard manure (3:1). Each plant was given 20 g urea, 25 g single superphosphate, and 12 g potassium sulfate 15 days after transplanting. Garden soil had electrical conductivity (EC) (1:2) = 0.32 dSm^{-1}, pH (1:2) 7.21, cation exchange capacity (CEC) = 10.63 Cmol kg^{-1}, and 0.33% organic carbon. The pots maintained in trays with test crops were then placed in Phytotron Growth Chamber and allowed to grow for 120 days with maintained RH from 55 to 65% during day and 75 to 90% in the darkness the Phytotron chamber (Model PGW 36) with growth area 3.3 m^2 (36 ft^2). The light was provided through 57 cool white fluorescent lamps having 25 W each located at about 1.5 m above the trays. The night temp. was maintained at 18°C, while day temp. at 25°C, 12 h period of daylight.

Percent change in plant height was measured using standard formula. Five matured leaves from three plants in each treatment were selected and photosynthec rate using an infrared gas analyzer (LI-6200, Li-Cor Biosciences, Lincoln, NE, USA). The leaf chlorophyll contents (chlorophyll a, b, and total chlorophyll) were estimated using the method suggested by Hiscox and Israelstam (6). The proline

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content in the matured leaves was estimated using a rapid colorimetric method (Bates *et al.*, 1). The method suggested by Luck (7) was followed to estimate the catalase activity, peroxidise by the method proposed by Thomas *et al.* (9). Analysis of variance (ANOVA) was performed for all experimental data and means were compared using the Duncan's multi-range test (DMRT) at 5% level of significance with SPSS.

It is well known that elevated CO_2 stimulates biomass production of C3 plants, and plants with indeterminate growth show higher growth enhancements in response to elevated CO_2 than plants with determinate growth, presumably because of differences in sink strength. Plants often show higher growth responses to elevated CO_2 when other resources such as nutrients and water are not limiting. Initial stimulations in growth in response to elevated CO_2 may diminish over time, possibly because of down-regulation of photosynthesis or modifications in biomass allocation and phenology. The percent increase in plant height as well as number of leaves was enhanced with increase in CO_2 concentration. The maximum increase in plant height and number of leaves were recorded in Kagzi Kalan (33.38% and 33.94) at 550 ppm CO_2 (Table 1). It has been shown that most of the plants show higher growth response to elevated CO_2 when other resources such as nutrients and water are not limiting. It was observed that under elevated CO_2 concentration, the biomass production of the two citrus genotypes showed higher growth enhancements (Norbert Keutgen and Kai Chen, 8). Initial stimulation in growth in response to elevated CO_2 may diminish over time, possibly due to down-regulation of photosynthesis or modifications in biomass allocation and phenology. The leaf CO_2 exchange rate of Kinnow plants (13.9 µmol CO_2 $m^{-2}s^{-1}$) was highest at 550 ppm CO_2 conc. as compared to Kagzi Kalan (12.1 µmol CO_2 m^{-2} s^{-1}), which increased with time in both the species. Leaf CO_2 exchange rate showed 16 to 98% higher at 800 ppm than at 400 ppm of CO_2 (Downton *et al.*, 4). Increase in photosynthetic rate is brought about by increased availability of CO_2 at the chloroplasts and reduction in photorespiration resulting from an increased ratio of CO_2 to O_2 (Farquhar and Sharkey, 5). The chlorophyll *a*, *b* and total chlorophyll parameters were maximum at an atmospheric CO_2 conc. of 550 ppm irrespective of genotype. However, maximum total chlorophyll contents (3.15 mg/g) in Kinnow and Kagzi Kalan (2.32 mg/g) were observed in 550 ppm CO_2 level. Total chlorophyll of leaves was independent of the CO_2 conc. in the growth chambers, which showed positive trends up to 550 ppm.

With the increase in CO_2 concentration in the growth chamber, CAT activity and proline level were enhanced in both the *Citrus* spp. (Table 2) Kinnow showed significantly higher CAT activity at 550 ppm (1.44 µmoles H_2O_2 hydrolyzed min^{-1} mg^{-1} protein) as compared to Kagzi Kalan (0.57 µmoles H_2O_2 hydrolyzed min^{-1} mg^{-1} protein) but minimum activity observed under control plants. The proline concentrations were recorded higher in Kinnow at 550 ppm (252.83 µg g^{-1}FW) as compared to Kagzi Kalan (83.72 µg g^{-1}FW) but lowest in control (91.33 and 55.50 µg g^{-1}FW in both genotypes. The POD activity irrespective of the genotype was relatively lower in Kagzi Kalan as compared to Kinnow. However, in Kinnow, it was very

Table 1. Effect of elevated CO_2 concentration on per cent increase in plant height, per cent increase in leaf number, photosynthesis rate, chl '*a*', chl '*b*' and total chlorophyll content in Kinnow mandarin and Kagzi Kalan lemon.

Treatment	Per cent increase in plant height	Per cent increase in leaf No.	Photosynthetic rate (µmol CO_2 m^{-2} s^{-1})	Chlorophyll '*a*' (mg/g FW)	Chlorophyll '*b*' (mg/g FW)	Total chlorophyll (mg/g FW)
Kinnow						
Control	6.09	12.61	10.4	1.63	0.73	2.33
450 ppm CO_2	6.80	14.20	11.2	2.2	0.80	3.07
550 ppm CO_2	17.06	22.92	13.9	2.2	0.89	3.15
Kagzi Kalan						
Control	12.48	17.80	9.6	1.3	0.38	1.66
450 ppm CO_2	25.64	19.81	11.7	1.5	0.44	1.89
550 ppm CO_2	33.38	33.94	12.1	1.7	0.65	2.32
CD ($P \leq 0.05$)						
Genotype (G)	2.8	5.5	NS	0.11	0.06	0.15
CO_2 level (L)	3.5	6.8	0.95	NS	0.07	0.18
G × L	4.9	9.6	1.33	NS	0.10	0.25

Table 2. Effect of elevated CO_2 levels on catalase and peroxidase activity, proline content and content in Kinnow mandarin and Kagzi Kalan lemon.

Treatment	Catalase (μ moles H_2O_2 hydrolyzed/min/mg^{-1} protein)	Proline content (µg/gFW)	Peroxidase (A_{436} unit min^{-1} µg g^{-1} FLW)
Kinnow			
Control	0.84	91.33	446.58
450 ppm CO_2	1.32	153.80	520.12
550 ppm CO_2	1.44	252.83	669.50
Kagzi Kalan			
Control	0.26	55.50	59.50
450 ppm CO_2	0.29	65.60	66.83
550 ppm CO_2	0.57	83.72	68.02
CD ($P \leq 0.05$)			
Genotype (G)	0.26	12.86	8.43
CO_2 level (L)	0.32	15.75	10.32
G × L	NS	22.28	14.6

NS = Non-significant

high (669.50 and 520.12 A_{436} unit min^{-1} µg g^{-1} FLW at 550 and 450 ppm, respectively) as compared to Kagzi Kalan (68.02 and 66.83 µg g^{-1} FLW at 550 and 450 ppm, respectively). Further, it was noticed that the peroxidise activity under both normal and high CO_2 conditions varied drastically and indicated difference in level of tolerance of the two citrus species against stress conditions.

This study suggested that among two citrus species, Kinnow showed more tolerance to high CO_2 conc. than Kagzi Kalan during short-term physiological responses of citrus to high CO_2 concentration. However, long term study are required for further assessment of elevated CO_2 on overall performance of different species.

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