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Effect of elevated CO_2 and temperature on phytochemistry of *Bt* cotton: Climate change perspective

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Abstract
A central issue in ecology is to determine how environmental variations associated with global climate change; especially changing atmospheric carbon dioxide (CO_2) and temperatures affect trophic interactions in various ecosystems, more so on the physiology of the plant system. In this regard, an investigation on phytochemistry of *Bt* cotton under different climate change treatments *viz.*, elevated CO_2 (550 ± 25 ppm) and temperature (2 °C rise) was conducted and compared with ambient levels of CO_2 and temperature under open top chambers (OTC's) at University of Agricultural Sciences, Raichur. The results indicated that climate change in terms of eCO_2 and temperature has favored the growth and development of *Bt* cotton crop, as it was evidenced by the accelerated growth rates in the form of plant height, number of leaves, leaf area, leaf area index (LAI), leaf water content (LWC) and sympodia. The growth parameters showed positive trend along with the yield parameters and resulted in increased yield in the eCO_2 and temperature treatments. Likewise, biochemical analysis of the *Bt* cotton plant showed lot of changes wherein, the chlorophyll content, carbon and carbon-based compounds *viz.*, tannins, phenols and sugars significantly increased in the eCO_2 conditions (CO_2 alone and in combinations with temperature) as compared to aCO_2 treatments. On the contrary, nitrogen (N) and N-based compounds *viz.*, proteins and amino acids decreased in the eCO_2 conditions which resulted in change in carbon to nitrogen (C: N) ratio. Climate change typically affected carbon and nitrogen dynamics in *Bt* cotton, which being a transgenic plant invests some nutrients for the production of specific toxic proteins (*i.e.*, endotoxins). This was evidenced in the present investigations which clearly showed that eCO_2 and temperature treatments decreased *Bt* toxin production

Keywords: Elevated CO_2, Elevated temperature, phytochemistry, *Bt* cotton

Introduction
Climatic variability, led by increase in atmospheric carbon dioxide (CO_2) and temperature do have a lot of implications on agriculture sector. In recent decade, climate change resultant global warming has become an issue of serious concern worldwide for existence of life on earth. According to Intergovernmental Panel on Climate Change (IPPC), it is defined as "Change in climate over time, either due to natural variability or as a result of human activity". In the near future, agriculture will inevitably face challenges caused by global climate change, which may be at global level and local level. It has been reported by federal agencies that CO_2 concentration has been increased by approximately 30 per cent since the industrial revolution which is believed to be responsible for an increase of about 0.66 °C in mean annual global surface temperature. Meanwhile, the temperature is anticipated to increase further by 1.4 to 5.8 °C by 2100 with equally increasing atmospheric CO_2. The atmospheric CO_2, which is considered to be chiefly responsible for the greenhouse effect, has increased from approximately 310 ppm in 1950 to about 400 ppm in the year 2011. This concentration is estimated to reach levels of 421 to 936 ppm by the end of 21st century, according to forecasting models, depending on the magnitude of future human activities (IPCC, 2013) [24]. Increased greenhouse gas concentrations in the atmosphere lead to physiological changes in plants. This topic has been extensively discussed and documented over past 20 years, especially with respect to modifications caused by the greenhouse gases of most concern, namely, CO_2 and O_3 (Ceulemans *et al.* 1999; Ashmore 2005; Lindroth 2010) [10, 6, 31]. The direct effects on plant included increased C:N ratio and increased growth rates (DeLucia *et al.*, 2012) [17], along with modifications in the production of plant metabolites (Pinto *et al.*, 2008) [36]. These direct effects on plants may also have indirect effects on organisms in the higher trophic systems, through the modification of trophic cascades (Bidart-Bouzat and Imeh-Nathaniel, 2008) [8].

So, to study the implications of climate change in terms of effect of both eCO_2 and temperature on phytochemistry, *Bt* cotton was chosen for the study which is a first transgenic C_3 plant in India and quite responsive to CO_2. Cotton (*Gossypium hirsutum* L.), popularly known as "White gold" is considered as an important fibre and commercial crop contributing about 85 per cent of raw material to textile industry, earning about 33 per cent of total foreign exchange (Anon., 2010) [3]. Globally, cotton is cultivated in an area of 29.8 million hectares with production of 25 million tonnes (Anon., 2014) [5], among which, India ranks first with an area of 11.61 million hectares and third in production with 6.2 million tonnes. By far, cotton is in commercial cultivation for domestic consumption and export needs in about 111 countries worldwide, hence called "King of fibres".

Material and Methods

In the climate change studies in agriculture, there are several approaches, but popularly Open Top Chambers (OTC's) are extensively used as plant exposure units both in air pollution and in CO_2 response studies in the field on crop plants. Currently, it is being used in North America, Europe and South Asian countries (Heagle *et al.*, 1973) [23].

In the present investigations, four circular OTC's with dimensions of 5 m diameter and 4 m height were constructed at Main Agricultural Research Station, University of Agricultural Sciences, Raichur and used for the study. Pure CO_2 mixed with ambient air was supplied to the chambers and maintained at set levels using manifold gas regulators, pressure pipelines, solenoid valves, rotameters, sampler, pump and CO_2 analyzer. The opening and closing of these valves were regulated on the basis of actual concentration of CO_2 within the OTC and the set CO_2 level for that particular OTC which was regulated by computer through linked Program Logic Control (PLC) and Supervisory Control and Data Acquisition (SCADA) system (www.neogenesisengg.com).

The temperature was maintained using infrared heaters mounted in OTC's two meters above canopy. The computer with uninterrupted power supply was established for uninterrupted data recording and storing. Each chamber was fitted with sensors to measure temperature and relative humidity and this facilitated the continuous monitoring of temperature, CO_2 and relative humidity. The data was continuously recorded for the temperature, relative humidity and actual CO_2 and displayed on the monitor continuously.

The eCO_2 and temperature (abiotic factors) were the main treatments in the present investigations. Each OTC was considered as a treatment for this study, which included:

T_1: Elevated CO_2 @ 550 ± 25 ppm

T_2: Elevated CO_2 @ 550 ± 25 ppm + rise in temperature of 2 °C

T_3: Ambient CO_2 @ 390 ± 25 ppm + rise in temperature of 2 °C

T_4: Ambient CO_2 @ 390 ± 25 ppm

T_5: Reference plot (Open plot)

Crop growing conditions

Bt cotton (MRC-7351) was sown during 2013 and 2014 in each OTC and reference plot in cement pots of size 42 × 32 cm containing mixture of FYM, vermicompost and soil in the ratio of 1:1:2. The soils filled in the pots were typical representative of Vertisol (black cotton soil). One plant was raised in each pot. Such pots were watered regularly and soon after the emergence, when seedlings were around 10 days old, each pot was covered with nylon cages of mesh size 0.15 × 0.15 cm so as to avoid infestation of insect pests. All the agronomic practices for raising the crop were practiced as per the package of practices of the University of Agricultural Sciences, Raichur (Anon, 2013) [4].

Plant growth parameters

Growth analysis is a mathematical expression of environmental effects on growth and development of crop plants and this is a useful tool in studying the complex interactions between the plant growth and the environment. Growth analysis in crop plants depends mainly on primary values (dry weights). The basic principle that underlie in growth analysis depends on two values (1) total dry weight of whole plant material (leaves, stems and reproductive structures) and (2) the total leaf area of the plant (specially chlorophyll content).

Measurement of plant growth was reflected in terms of plant height, number of leaves, sympodia and leaf area was recorded at 30, 60, 90 and 120 days of crop growth during each year separately and later two seasons data was pooled and presented. Plant height, number of leaves and sympodia were recorded manually whereas, leaf area was measured by hand planimeter.

This data was used to calculate various indices and characteristics that describe the growth of the plant and of their parts grown in different environments and the relationship between assimilatory apparatus and dry matter production. Later, these parameters were used to calculate growth indices like leaf water content (%) and leaf area index (LAI).

Biochemical analysis

Biochemical analysis was carried out by taking leaf samples at 30, 60, 90 and 120 days of crop growth in each year/ season from the OTC's under elevated and aCO_2 conditions as well as reference plot, and analyzed. Organic carbon, leaf nitrogen, C: N ratio, chlorophyll, tannins, phenols, reducing and total sugars, soluble proteins and free amino acids were analyzed as per the standard procedures. Later the data from all the stages was pooled and presented.

Total organic carbon was estimated by dry combustion method/ash method using Muffle furnace whereas, leaf nitrogen in the plant sample was determined by using Micro-Kjeldahl technique, discovered by McKenzie (1994) [34]. Carbon to nitrogen ratio was calculated by taking the ratio of carbon and nitrogen content readings priorly obtained by estimation of the same.

Leaf chlorophyll were measured using a Dualex Scientific sensor, a hand tool leaf clip combining the use of fluorescence as well as light transmission of a leaf to determine its physiological status. Sugars, phenols and tannins were estimated by methods suggested by Nelson Somogyi (Marais *et al.*, 1996) [33]. Phenols were estimated by Folin - Ciocalteau (FCR) method while tannins were estimated by Folin-Dennis method (Malick and Singh, 1980) [32]. Soluble proteins were estimated by Lowry's method while colorimetric estimation of total free amino acids was carried out by Ninhydrin method introduced by Moore and Stein (1948) [35].

Bt delta endotoxin (Cry 1Ac and Cry 2Ab2) estimation was carried out by ELISA method to quantify *Bt* toxin expression at different stages.

Statistical analysis

Data on present investigations were statistically analyzed using one-way analysis of variance (ANOVA). Variable treatments and replications were taken for different studies so as to suit the requirements of the experiment. The differences between mean values were compared with least significant difference at both $p< 0.01$ and 0.05. All statistical analysis were done using SPSS version 16.0, a statistical software tool.

Results

Growth and yield parameters

Elevated CO_2 and temperature had significantly influenced the plant growth and development wherein, eCO_2 (550 ppm) treatment recorded more number of leaves (67.58/plant) and plant height (79.72 cm) which was on par with eCO_2 + eTemperature (550 ppm+2°C) treatment (67.53/plant and 78.73 cm). Whereas, the same parameters recorded by the ambient treatments was less wherein, the aCO_2 + eTemperature (390 ppm + 2°C) treatment recorded least (54.84 leaves/plant and 64.77 cm plant height) which was on par with aCO_2 (390 ppm) alone (55.98 leaves/plant and 66.13 cm plant height) and reference plot (56.13 leaves/plant and 67.70 cm), respectively (Table 1).

In the same way, the sympodia recorded in the eCO_2 treatments were highest wherein, eCO_2 (550 ppm) alone recorded 12.61 per plant which was on par with eCO_2 + eTemperature (550 ppm + 2 °C) treatment (12.48/plant). Meanwhile, the least sympodia was recorded in the aCO_2 treatments wherein, aCO_2 (390 ppm + 2 °C) treatment recorded 9.63 per plant and aCO_2 (390 ppm) recorded 9.98 per plant, respectively. Likewise, the seed cotton yield recorded was significantly higher in both eCO_2 levels wherein, eCO_2 (550 ppm) recorded 321.67 gram per plant which was on par with eCO_2 + eTemperature (550 ppm + 2 °C) treatment (315.32 g/plant). Whereas, the aCO_2 treatments recorded minimum seed cotton yield wherein, the reference plot registered 253.31 gram per plant which was non-significant with the rest of the aCO_2 treatments. The per cent increase in the seed cotton yield noticed in the eCO_2 treatments was 21.25 per cent in eCO_2 (550 ppm) treatment while eCO_2 + eTemperature (550 ppm + 2 °C) treatment recorded 19.66 per cent increase when compared to the standard check (reference plot) (Table 1).

Growth indices

The growth indices *viz.*, leaf area, leaf area index (LAI) and leaf water content recorded at various growth stages showed a significant difference among the CO_2 treatments. The leaf area was the highest (289.94 dm^2/plant) in the eCO_2 (550 ppm) treatment which was followed by eCO_2 + eTemperature (550 ppm + 2°C) treatment (285.18 dm^2/plant) which was on par with the latter treatment. Similarly, as the leaf area increased, the LAI also increased in the eCO_2 treatments. The highest LAI (5.31) was noticed in the eCO_2 (550 ppm) treatment which was on par with the eCO_2 + eTemperature (550 ppm + 2 °C) treatment (5.23). However, the leaf water content was significantly less in the elevated treatments which recorded 82.87 per cent and 82.28 in the eCO_2 (550 ppm) treatment and eCO_2 + eTemperature (550 ppm + 2 °C) treatments respectively. However, the reference plot recorded the highest leaf water content (88.29%) which was on par with the rest of the aCO_2 treatments (Table 2).

Biochemistry

Chlorophyll

Significant variation in leaf pigments was noticed among different climate change treatments wherein, the chlorophyll content recorded was the highest (36.49 μg/cm^2) in eCO_2 (550 ppm) alone but showed non-significant difference with eCO_2 + eTemperature (550 ppm + 2°C) treatment (34.44 μg/cm^2). Whereas, aCO_2 + eTemperature (390 ppm+2°C) treatment recorded least chlorophyll content (32.10 μg/cm^2) which was on par with aCO_2 (390 ppm) treatment which recorded 32.35 μg/cm^2 and reference plot with 32.60 μg/cm^2 (Table 3).

Leaf nitrogen, carbon and C:N ratio

Maximum leaf nitrogen of 5.02 per cent was recorded in reference plot which was followed by aCO_2 (390 ppm) alone (5.00%) and with 2°C rise in temperature treatment (4.99%). On the contrary, there was a significant increase in per cent carbon and was noticed in eCO_2 (550 ppm) alone and in combination with increased temperature as compared to aCO_2 treatments. Highest value of carbon (48.39%) was noticed in eCO_2 (550 ppm) treatment which was on par with eCO_2 + eTemperature (550 ppm + 2 °C) treatment (48.24%) and least was recorded in aCO_2 + eTemperature (390 ppm + 2°C) (42.98%) treatment. Due to increase in carbon and decrease in nitrogen levels in the eCO_2 conditions, the C: N ratio increased accordingly. It ranged from 8.90 to 11.51 across the treatments, in which eCO_2 (550 ppm) treatment showed highest C: N ratio (11.51) and reference plot recorded least C: N ratio (8.90) (Table 3).

Tannins and phenols

Tannins and phenols were significantly affected by the eCO_2 and temperature treatments. Elevated CO_2 @ 550 ppm alone recorded highest tannin (4.04 mg/g) and phenol (5.20 mg/g) content which was non-significant with eCO_2 + eTemperature (550 ppm + 2 °C) treatment which recorded 3.97 and 5.14 mg/g of tannins and phenols, respectively. However, the aCO_2 treatments showed low tannin and phenol content wherein, aCO_2 + eTemperature (390 ppm + 2 °C) treatment recorded least tannin (3.01 mg/g) and phenol (3.82 mg/g) content followed by aCO_2 (390 ppm) treatment and reference plot (Table 3).

Total Sugars and Reducing Sugars

Total and reducing sugars had a significant positive effect of eCO_2 and temperature treatments. The sugars were high in the eCO_2 levels as compared to aCO_2 levels wherein, the eCO_2 (550 ppm) treatment recorded highest values for total sugars (5. 92mg/g) and reducing sugars (4.11 mg/g) and the least was recorded by aCO_2 + eTemperature (390 ppm + 2 °C) treatment (4.87 and 3.00 mg/g) (Table 3).

Soluble proteins and Free Amino Acids

Proteins as well as amino acids showed significant decrease in the eCO_2 conditions. The highest protein (4.88 mg/g) and amino acid (0.73 mg/g) content was recorded in reference plot which was on par with the aCO_2 (390 ppm) treatment and aCO_2 + eTemperature (390 ppm + 2 °C) treatments. Meanwhile, the eCO_2 conditions recorded least values of soluble proteins and free amino acids wherein, the eCO_2 (550 ppm) treatment recorded 4.25 mg/g of proteins and 0.59 mg/g of amino acids (Table 3).

***Bt* toxin:** *Bt* toxin expression was quantified by ELISA method in leaves, squares and boll rind in all the climate change treatments to know the effect of eCO_2 and temperature on the toxin expression. Present investigations have clearly showed that eCO_2 and temperature treatments (550 ppm alone and in combination with 2 °C more temperature) have decreased *Bt* toxin production (Cry1Ac of 1.05 µg/g of leaves and Cry2Ab2 of 63.18 µg/g of leaves) compared to aCO_2 and temperature treatments (Cry1Ac of 1.17 µg/g of leaves and Cry2Ab2 of 68.05 µg/g of leaves) at 90 DAS. The toxin expressed and quantified from squares and boll rind followed the same trend as above wherein, the aCO_2 treatments recorded highest Cry1Ac (0.21 µg/g of squares; 1.45 µg/g of boll rind) and Cry2Ab2 (61.91 µg/g of squares; 51.18 µg/g of boll rind) while the eCO_2 and temperature treatments recorded the least Cry1Ac (0.17 µg/g of squares;1.27 µg/g of boll rind) and Cry2Ab2 (57.93 µg/g of squares; 48.59 µg/g of boll rind). There was a decrease in toxin (25.64% of Cry1Ac in leaves, 23.52% in squares and 14.13% in boll rind; 7.70% of Cry2Ab2 toxin in leaves, 6.87% in squares and 5.33% in boll rind) as compared to standard check (reference plot) (Fig. 1 and 2).

Table 1: Effect of elevated CO_2 and temperature on growth, yield parameters and yield of *Bt* cotton

Treatment	Leaves/plant	Plant height (cm)	Sympodia/plant	Seed cotton yield (g/plant)	% increase or decrease in yield over standard check
eCO_2 (550 ppm)	67.58[a]	79.72 [a]	12.61 [a]	321.67 [a]	21.25
eCO_2 + eTemp. (550 ppm + 2 °C)	67.53 [a]	78.73 [a]	12.48 [a]	315.32 [a]	19.66
aCO_2 + eTemp. (390 ppm + 2 °C)	54.84 [b]	64.77 [b]	9.63 [b]	261.46 [b]	3.21
aCO_2 (390 ppm)	55.98 [b]	66.13 [b]	9.98 [b]	254.21 [b]	0.35
Reference plot (Open plot)	56.13 [b]	67.70 [b]	10.15 [b]	253.31 [b]	--
$F_{(4,15)}$	27.28**	26.11**	159.2**	44.89**	--
S.Em ±	0.56	0.71	0.12	3.87	--
CD (p=0.01)	2.33	2.96	0.51	16.12	--

**Significant @ 1%
Means denoted by same letters in vertical column are not significantly different by DMRT
Note: Data presented in the table is pooled data of two seasons

Table 2: Effect of elevated CO_2 and temperature on growth indices of *Bt* cotton

Treatment	Leaf area (dm^2/plant)	Leaf area index (LAI)	Leaf water content (%)
eCO_2 (550 ppm)	289.94 [a]	5.31 [a]	82.87 [a] (65.55)
eCO_2 + eTemp. (550 ppm + 2 °C)	285.18 [a]	5.23 [a]	82.28 [a] (65.10)
aCO_2 + eTemp. (390 ppm + 2 °C)	237.95 [b]	4.36 [b]	87.05 [b] (68.90)
aCO_2 (390 ppm)	240.10 [b]	4.38 [b]	87.89 [b] (69.63)
Reference plot (Open plot)	245.33 [b]	4.50 [b]	88.29 [b] (69.98)
$F_{(4,15)}$	9.49**	16.21**	8.23**
S.Em ±	2.28	0.04	0.51
CD (p=0.01)	9.48	0.17	2.15

**Significant @ 1%
*Figures in parentheses are arcsine transformed values
Means denoted by same letters in vertical column are not significantly different by DMRT
Note: Data presented in the table is pooled data of two seasons

Table 3: Effect of elevated CO_2 and temperature on phytochemistry of *Bt* cotton

Treatments	Biochemical constituents									
	Chlorophyll (µg/cm²)	Leaf nitrogen (%)	Carbon (%)	C:N ratio	Tannins (mg/g)	Phenols (mg/g)	Total sugars (mg/g)	Reducing sugars (mg/g)	Soluble proteins (mg/g)	Free amino acids (mg/g)
eCO_2 (550 ppm)	36.49 [a]	4.33 [b] (12.01)	48.39 [a] (44.07)	11.51 [a]	4.04 [a]	5.20 [a]	5.92 [a]	4.11 [a]	4.25 [b]	0.59 [b]
eCO_2+eTemp. (550 ppm + 2 °C)	34.44 [a]	4.38 [b] (12.08)	48.24 [a] (43.99)	11.44 [a]	3.97 [a]	5.14 [a]	5.79 [a]	4.10 [a]	4.27 [b]	0.60 [b]
aCO_2 +eTemp. (390 ppm + 2 °C)	32.10 [b]	4.99 [a] (12.90)	42.98 [b] (40.96)	8.98 [b]	3.01 [b]	3.82 [b]	4.87 [b]	3.00 [b]	4.85 [a]	0.71 [a]
aCO_2 (390 ppm)	32.35 [b]	5.00 [a] (12.92)	43.24 [b] (41.11)	8.92 [b]	3.03 [b]	3.86 [b]	4.92 [b]	2.98 [b]	4.85 [a]	0.72 [a]
Reference plot (Open plot)	31.60 [b]	5.02 [a] (12.94)	43.66 [b] (41.35)	8.90 [b]	3.06 [b]	3.88 [b]	4.92 [b]	3.07 [b]	4.88 [a]	0.73 [a]
$F_{(4,15)}$	17.62**	25.67**	129.3**	183.7**	105.9**	124.2**	73.5**	58.9**	9.02**	19.2**
S.Em ±	0.26	0.04	0.19	0.08	0.01	0.02	0.03	0.03	0.04	0.01
CD (p=0.01)	1.08	0.16	0.78	0.32	0.06	0.10	0.11	0.11	0.16	0.03

**Significant @ 1%
Figures in parentheses are arcsine transformed values
Means denoted by same letters in vertical column are not significantly different by DMRT
Note: Data presented in the table is pooled data of two seasons

Discussion

Plant growth parameters

Plant growth was enhanced in eCO_2 conditions as compared to aCO_2 which was noticed in terms of significant increase in number of leaves and plant height in elevated CO_2 conditions as compared to ambient treatments over the cropping period. These results indicated that the growth has increased in the elevated conditions which may be reasoned to the fact that the CO_2 has a direct fertilizing effect on the plant growth. The plants in the elevated treatments have utilized much CO_2 and resulted in increased growth. Cotton, being a C_3 plant has found to show increased growth rates in the elevated CO_2 conditions and the present studies on growth parameters are well supported by various studies who have reported similar results wherein, increased growth rates of cotton in the elevated CO_2 treatments was noticed (Wong, 1979; Leasley and Fakhri, 2001; Gang *et al.*, 2007b) [29, 44, 19].

Growth indices, especially leaf area and leaf area index (LAI) values were highest in elevated conditions of CO_2 alone and in combination with temperature which showed their significance over aCO_2 levels. The results from the present investigations revealed that the eCO_2 and temperature have influenced positively on growth indices *viz.*, leaf area and leaf area index whereas, the leaf water content was decreased in the eCO_2 conditions as compared to aCO_2 conditions. The decrease in leaf water content in the elevated treatments may be reasoned to the increase in the leaf surface temperature and evapotranspiration. Many of earlier studies are in line with the present findings which reported that cotton being a C_3 plant, responds positively to CO_2 showing increased growth rates (Wong, 1979) [44]. The direct effects of eCO_2 on individual plant species have been well documented (Curtis and Wang, 1998) [15] wherein, eCO_2 generally resulted in increased leaf area, leaf area index and growth rate. They also recorded that leaf area of transgenic *Bt* cotton increased when exposed to CO_2 gas for three successive months under two CO_2 levels while, the leaf water content decreased in the elevated levels.

Some other studies which support our present findings revealed that cotton crop grown in eCO_2 had greater leaf area (Leasley and Fakhri, 2001) [29] and higher leaf photosynthesis, non-structural carbohydrates, and total biomass (Raja and Zhao, 2005) [38] as compared to aCO_2 (Zhao *et al.*, 2003) [49]. More plant height, leaves and more number of fruiting branches were recorded in the eCO_2 treatments than in the aCO_2 levels as the former treatments have decrease in leaf water content in cotton bolls as reported by Gang *et al.* (2007b) [19]. Similarly, in some other crops like lucerne (James *et al.*, 2014) [25] and legume (Jennifer *et al.*, 2008) [26] more plant height was noticed under eCO_2 and temperature conditions.

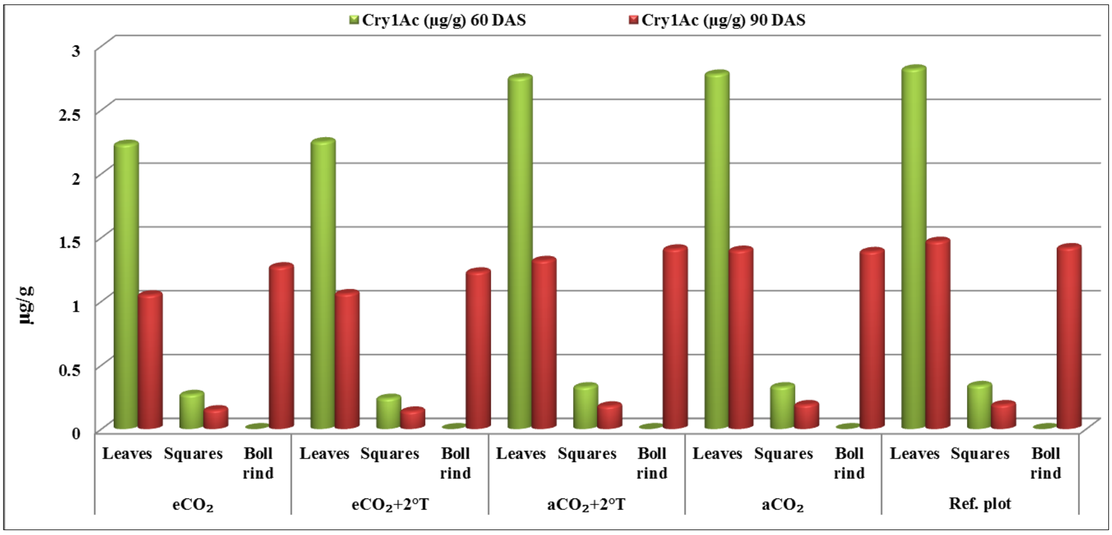

Fig 1: Effect of elevated CO_2 and temperature on Cry 1 Ac *Bt* toxin concentration

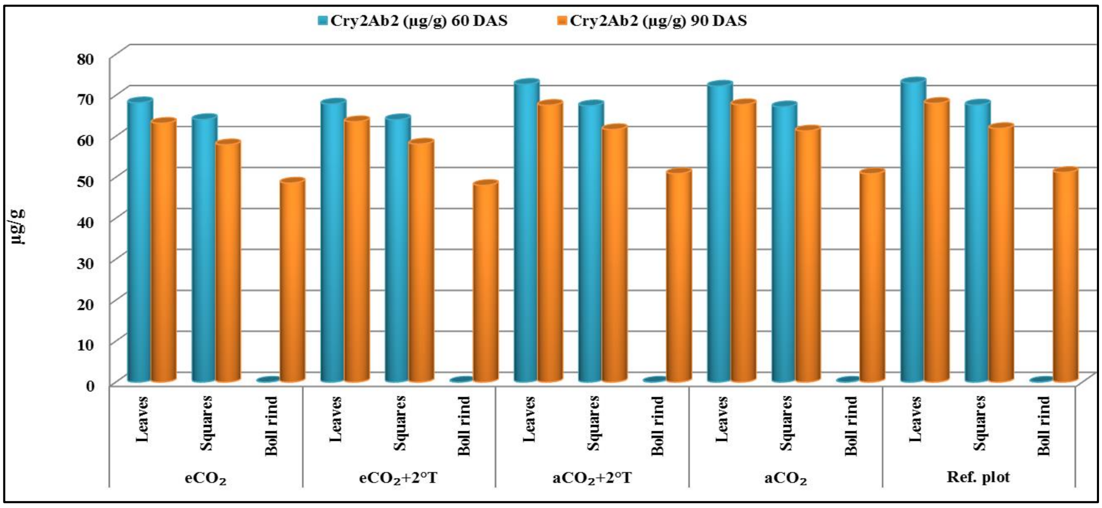

Fig 2: Effect of elevated CO_2 and temperature on Cry 2 Ab2 *Bt* toxin concentration

Yield parameters and yield

Results of present investigations showed significant increase in the yield parameters and yield in the eCO_2 conditions as compared to aCO_2 conditions. More number of sympodial branches with more bolls has resulted in substantial increase in yield in the elevated climate change treatments. Various studies are in line with the present findings with respect to yields which reported that yields of C_3 agricultural crops are estimated to increase by about 30 per cent if CO_2 is doubled (Kimball, 1983) [28]. Likewise, increased aboveground biomass and yield of tobacco (Xue *et al.*, 2010; Leasley and Fakhri, 2001) [47, 29] and lucerne (James *et al.*, 2014) [25] under eCO_2 concentration was recorded.

Biochemistry

Biochemical analysis was carried out to know the changes in phytochemistry as affected by eCO_2 and temperature. The plant physiological and biochemical constituents *viz.*, chlorophyll, carbon, tannins, phenols and sugars showed significant increase while, nitrogen, proteins and amino acids decreased in the eCO_2 conditions.

Chlorophyll

The chlorophyll content showed an increased trend in the elevated climate change treatments, wherein they showed more values. This increase in the chlorophyll content may be reasoned to the much utilization of carbon in the tissues which inturn has increased the chlorophyll content. More the chlorophyll, more the photosynthesis and growth rate in the elevated climate change treatments, which was evident by the increase in the growth and yield parameters which was mentioned in the previous headings of the same. The rise in chlorophyll content in the elevated climate change treatments was reported by many studies in various crops. Studies of numerous authors are in line with the present findings wherein, they reported that plant chlorophyll content rose up with increased CO_2 concentration, especially in C_3 plants (Hamid *et al.*, 2012) [21]. The studies by Sari *et al.* (2008) [41] showed increased chlorophyll content in *Bt* oilseed rape under eCO_2 treatments as compared to ambient.

Leaf nitrogen, carbon and C:N ratio

The present findings indicated that the leaf nitrogen has certainly decreased in the elevated climate change treatments as compared to ambient treatments. These data on present investigations on phytochemistry showed that, transgenic *Bt* cotton is able to shift the allocation between N-based and C-based defensive compounds, which may depend on the changes in relative availability of carbon and nitrogen inputs in eCO_2. It is well known that the direct fertilizing effects of enriched atmospheric CO_2 on plant growth, physiology and community structure is depicted by increased plant growth by accelerating rates of photosynthesis, which reduces tissue quality and increases the carbon to nitrogen (C: N) ratio (Robinson *et al.*, 2012) [40]. Elevated CO_2 increases biomass, reduces foliar nitrogen and increases C: N ratio for most plants, especially C_3 crops (Chen *et al.*, 2005a) [16]. Likewise, eCO_2 generally decreases nitrogen concentrations and increases phenolic and carbohydrate concentrations and C: N ratios (Lindroth, 2010) [31] but, decline in leaf nutritional quality (especially N). Elevated CO_2 decreased nitrogen content and increased C: N ratio of transgenic and non-transgenic *Bt* oilseed rape plant types. Elevated temperature increased carbon and nitrogen contents under aCO_2, but decreased these under eCO_2 as reported by Sari *et al.* (2008) [41]. Some more studies are discussed in the light of our present investigations. A study by Carlos *et al.* (2000) [9] on cotton plants (*Gossypium hirsutum* L.) that were grown under two CO_2 concentrations *viz.*, aCO_2 (300 µmol mol^{-1}) and eCO_2 (900 µmol mol^{-1}) inside the environmental chambers showed that there was a strong CO_2 effect on nitrogen content in plants. Plants grown under eCO_2 showed decrease in nitrogen content compared to plants grown in aCO_2 atmosphere whereas, the C: N ratio of plant was significantly increased under eCO_2 than aCO_2 (Coviella *et al*., 2002) [14]. Cotton crop showed increase in C: N ratio of foliage and decrease in nitrogen, primarily because of accumulation of non-structural carbohydrates (Lindroth *et al.*, 1995) [30] and the transgenic plants grown in eCO_2 produced lower levels of *Bt* toxin than those grown in aCO_2.

Apart from cotton, the foliar chemistry studied under eCO_2 for other crops are also taken in support to our present findings. Low nitrogen and high carbon content with increased C: N ratio was noticed but, no change in phenol content in chickpea grown under eCO_2 (550 and 700 ppm) (Abdul *et al.*, 2014) [1]. The reports of Srinivasa *et al.* (2009) [42] revealed the effect of eCO_2 on leaf quality of Castor (*Ricinus communis* L.) and reported that, leaf nitrogen content was distinctly lower in eCO_2 foliage while, carbon content was higher and consequently, the change in the relative proportion of carbon to nitrogen (C: N ratio) was considerably higher in eCO_2 foliage.

Tannins and phenols

The present findings on tannins and phenols revealed that the elevated conditions have well affected these defensive chemicals wherein, highest of these were found in the elevated treatments as compared to ambient levels. Since, the tannins and phenols are carbon-based compounds, along with increase in the carbon content, these compounds have also tend to increase in the elevated climate change treatments. These findings are in line with studies of Xiaowei *et al.* (2008) [45] who reported that some kinds of defensive secondary components such as phenolics tend to increase in the eCO_2 conditions. Further, eCO_2 foliage had higher polyphenols content too, compared to aCO_2 foliage as reported by Coll and Hughes (2008) [13].

Sugars

The results on the present investigations on plant sugars showed they varied significantly across elevated and aCO_2 and temperature treatments. The highest total sugars and reducing sugars were recorded in eCO_2 (550 ppm) treatment which was on par with eCO_2 + eTemperature (550 ppm + 2°C) treatment. However, the aCO_2 treatments recorded low sugars as compared to eCO_2 treatments. The studies by Ramachandra *et al.* (1998) [39] are in line with the present findings which showed that starch and sucrose concentrations were always high in leaves grown under eCO_2 when compared to ambient treatments. Likewise, Sari *et al.* (2008) [41] also recorded more sugars in *Bt* oilseed rape plant under eCO_2 conditions than ambient conditions. Studies by Xin *et al.* (2013) showed that eCO_2 increased carbohydrates accumulation in tomato plants and the leaf carbohydrate determinations showed that the starch, total soluble sugar, and sucrose concentrations increased significantly in plants exposed to eCO_2.

Soluble proteins and free amino acids

The present investigations showed that soluble proteins and amino acids had a significant negative effect of eCO_2 and temperature treatments. Elevated CO_2 treatments recorded low proteins and amino acids while the aCO_2 treatments recorded highest values. Studies in line with the present findings reported similar results wherein, lower amounts of amino acids were found in *Bt* cotton phloem under eCO_2 than under aCO_2 levels as reported by Sun *et al.* (2009) [43] and Chen *et al.* (2004) [7] in spring wheat. Similarly, Yin *et al*. (2010) [48] reported that atmospheric CO_2 enrichment induced changes in phytochemistry of maize plant which decreased protein and total amino acids.

Overall, eCO_2 can affect plant quality by inducing changes in allocation to primary and secondary metabolites (Agrell *et al.*, 2000; Hartley *et al.*, 2000; Goverde and Erhardt, 2003) [2, 22, 20]. Many studies have shown that eCO_2 increases net photosynthesis in C_3 plants because higher CO_2 can suppress RuBP oxygenase activity; decrease photorespiration; and increase carbon assimilates for plant growth and development. Elevated CO_2 accelerates the photosynthetic rate, stimulates plant growth, and increases the carbon: nitrogen ratio of most plant species (Poorter *et al.*, 1997; Curtis and Wang, 1998; Barbehenn *et al.*, 2004) [37, 15]. Likewise, results of the study on spring wheat (*Triticum aestivum* L.) revealed that the host plants grown at eCO_2 (550 and 700 ppm) generally had greater starch, sucrose, glucose, total non-structural carbohydrates (TNCs), free amino acids, soluble protein and less fructose and nitrogen as reported by Chen *et al*. (2004) [7].

Different crop species react differently to eCO_2 and temperature conditions wherein, most of the crops have showed increase in carbon assimilates, sugars while nitrogen, proteins and amino acids were decreased while, some crops have showed increased levels of these secondary metabolites. These studies show that the increase and decrease of these metabolites merely depends on the responses of the crop species to the elevated levels of atmospheric CO_2 and temperature.

***Bt* toxin**

Climate change factors such as eCO_2 and temperature typically affect carbon and nitrogen dynamics of crop plants and the performance of insect herbivores. Insect-resistant transgenic plants invest some nutrients to the production of specific toxic proteins (*i.e.*, endotoxins from *Bacillus thuringiensis*) which could alter the C-N balance of these plants, especially under changed abiotic conditions (Gang *et al.*, 2007a) [18].

The present investigations on the effect of eCO_2 and temperature on *Bt* toxin has revealed that the toxin expression has decreased with the increase in CO_2 concentration which are in line with the studies of Carlos *et al.* (2000) [9]. Present results are in conformity with some of the studies which are discussed wherein, *Bt* toxin decreased by 4 per cent and 2.5 per cent in transgenic *Bt* cotton in two consecutive years which is presumed that eCO_2 (double-ambient) can alter the plant growth and ultimately the phenotype allocation to foliar chemical components of transgenic *Bt* cotton, which may in turn affect the plant-herbivore interactions (Gang *et al.*, 2007b) [19].

High temperature also had effect on the insecticidal properties of *Bt* cotton which resulted in the degradation of soluble proteins in the leaf, which resulted in decline of the level of the toxin Cry1A (Chen *et al.*, 2005) [16]. These proteins are nitrogen-based defenses that have a major impact on several common insect pests, greatly reducing yield losses (Dehua *et al.*, 2005) [16]. It was also reported that growing these transgenic plants in eCO_2 resulted in a nearly 25 per cent reduction of the expression of these proteins (John and Cassey, 2009) [27].

In conclusion, the climate change in terms of eCO_2 and temperature has favoured the growth and development of *Bt* cotton crop, as it was evidenced by the accelerated growth rates and increased yield in the eCO_2 and temperature treatments. Phytochemistry of the *Bt* cotton plant was expidated and it showed lot of changes wherein, the carbon-based compounds significantly increased while, N-based compounds decreased in the eCO_2 conditions (CO_2 alone and in combinations with temperature) as compared to aCO_2 treatments. Climate change typically affected carbon and nitrogen dynamics in *Bt* cotton, which is a transgenic plant that invests some nutrients for the production of specific toxic proteins (*i.e.,* endotoxins) and for this matter, present investigations have clearly showed that eCO_2 and temperature treatments have decreased *Bt* toxin production.

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