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IMPACT OF HIGH-ELEVATED CO2 CONCENTRATION ON CAROTENOIDS AND PROLINE CONTENT OF ARACHIS HYPOGAEA (L)

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ABSTRACT

Increasing atmospheric CO_2 concentration leads environmental changes on the earth; it causes different alterations in plant growth and production. Groundnut is a very important edible oilseed crop raised mostly under rain fed situations worldwide. The present investigation was conducted during 2014-2015 in randomized controlled chamber at botanical garden in Sri Venkateswara University. To evaluate the effects of normal CO_2 level and elevated concentration levels on *Arachis hypogaea* (L) with different concentrations of CO_2 levels (400ppm, 600ppm, 800ppm,) to investigate the total carotenoids and total proline content was measured in days of 5^{th}, 10^{th} and 15^{th} intervals. The results showed that in *Arachis hypogaea* (L) the elevated CO2 enhance the carotenoids and proline content significantly with increasing concentration of CO2.

KEYWORDS: Elevated CO2, Arachis hypogaea, total carotenoids, proline.

INTRODUCTION

Global atmospheric concentrations of greenhouse gases (e.g., CO_2 and O_3) have increased due to human being activities since industrialization. Carbon dioxide is naturally present in the atmosphere as part of the Earth's carbon cycle, enhanced atmospheric CO_2 concentration influence the functions of ecosystem including agricultural yield. It is one of the most important greenhouse gases linked to global warming, but it is a minor component of Earth's atmosphere (about 3 volumes in 10,000), formed in combustion of carbon-containing materials (www.britannica.com/science). The presence of the gas in the atmosphere keeps some of the radiant energy received by Earth from being returned to space, thus producing the so-called greenhouse effect (*Yan et al., 2018*). Global monthly average concentration of CO2 have risen from 339 ppm average in 1980 to 405 ppm in 2018, it was increase of more than 20% of carbon dioxide. It is the primary greenhouse gas emitted through anthropogenic activities, at the end of the 21st century it may reach even up to 750 to 1000 ppm (globalchange.gov). responses of crop to climate change are closely related to the local climate therefore, crop responses to climate change different with region and plant species (IPCC 2007). CO_2 is a plant nutrient and its enrichment expected to enhance crop growth and productivity by increasing photosynthesis and water use efficiency and lowering transpiration because of minimize the conductance of stomata (*long at al*., 2004; *ziska and bunce* 2006). *Heineman at all* (2006) reported that elevated CO_2 generally increase the plant biomass volume and length of root and shoot, these parameters shows that plant has high response in low temperature low response in high temperature with increasing CO_2 concentration.

Peanut (*Arachis hypogaea* L.), also known as groundnut, earthnut, and ground bean, It is the world's fourth most important source of edible vegetable oil and third most important source of vegetable protein. used in cooking, margarines, salads, canning for deep-frying, pharmaceuticals, soaps, cold creams, pomades lubricants, emulsions for insect control, India, Nigeria, U S A29922, China and Myanmar are the major peanut growing countries. India is the second largest producer of peanut in the world, with an average annual production of 5.51 million tons (http://faostat.fao.org). The oil cake of ground nut has high-protein using for livestock feed and may be used for human consumption (Sanders et al., 2003). elevated CO2 in the atmosphere is influence the plant photosynthetic rate, physiological growth parameters of biomass and pod yield of groundnut (*stanciel at al*., 2000). Being c3 plants legumes have more potential to improve the benefit of elevated CO_2 by stimulate the photosynthesis with increased N_2 fixation (*rogers at al*., 2009). a meta analysis of environmental changes on heat ,ozone and co2 concentrations that highly impact the nutritional quality of vegetables and legumes (*Pauline at al*., 2018). According to *Vaidya et al*., 2014, clearly reveals that elevated CO2 conditions enhanced the plant growth and total biomass in different genotypes of arachis hypogaea butt impact of elevated CO_2 alteration are not same in different genotypes. (Genotypes- JL-24, ICGV 91114, Narayani, Abhaya, Dharani). Nutritional point of view elevated CO2 concentrations significantly increased the omega 6 and omega 9 fatty acids in peanuts and improve yield and kernel quantity (*sk yadav et at.*, 2011). In this respect, the present work evaluating the variation of carotenoids and proline concentration in Arachis hypogaea L (mg/g.fr.wt) with different concentration of CO_2 such as 400ppm, 600ppm, 800ppm.

MATERIAL AND METHODS

Seed materials

The certified seeds of *Arachis hypogaea* .L were purchased from N. G. Ranga Agriculture College Tirupati, Chittoor district, Andhra Pradesh. Seeds with uniform size, color and weight were chosen for the experimental purpose.

Experimental conditions and CO2 exposure

Experimental plants *Arachis hypogaea* L. grown in pots (33 cm diameter × 23 cm depth) during 2014-2015 in randomized complete block design (RCBD) CO_2 chamber with four replications at botanical garden in Sri Venkateswara University, Tirupati (latitude 13.6288° N, longitude 79.4192° E), Andhra Pradesh , India. (Each closed top chamber has 2.4 m in diameter 2.5 m in height).Seeds of *Arachis hypogaea* (L.) were soaked overnight in distilled water at 25.8^C, after which they were transferred to Petri dishes containing filter paper moistened with distilled water. seeds were germinated under either ambient or elevated CO_2 for seven days and then transferred to the pots. plants were maintained in a controlled-climate and ambient growth and photoperiod for a week to allow plant acclimation to the growth chamber conditions. The CO_2 concentration was manipulated automatically by controlling the amount of CO_2 gas injected, as described previously (*Lee et al.*, 2000). Twenty days old seedlings were progressively exposed to 400 ppm, 600 ppm and 800 ppm concentration continued for two weeks for two hours with one-day leave. The air temperature, air relative humidity, light and day lengths were optimum for the growth of *Arachis hypogaea* (L.). leaves were harvested to estimate the total carotenoids and proline content.

Table 1: Variation of carotenoids concentration in *Arachis hypogaea*L. (mg/g.fr.wt) with different concentration of CO_2.

Name of the plant	Concentration of CO_2	5th day	10th day	15th day
*Arachis hypogaea*L.	Control	0.320±0.016	0.421±0.210	0.541±0.270
	400 ppm	0.485±0.024	0.954±0.047	1.245±0.062
	600 ppm	0.558±0.027	1.754±0.087	1.954±0.097
	800 ppm	1.251±0.062	2.35±0.117	3.214±0.160

Values represented in table are means of three replicates ± SE (Standard Error) Values in the column bearing one or more than one common letters in superscript are not significantly different from each other (P ≤ 0.05) according to DMR (Duncan Multiple Range) test.

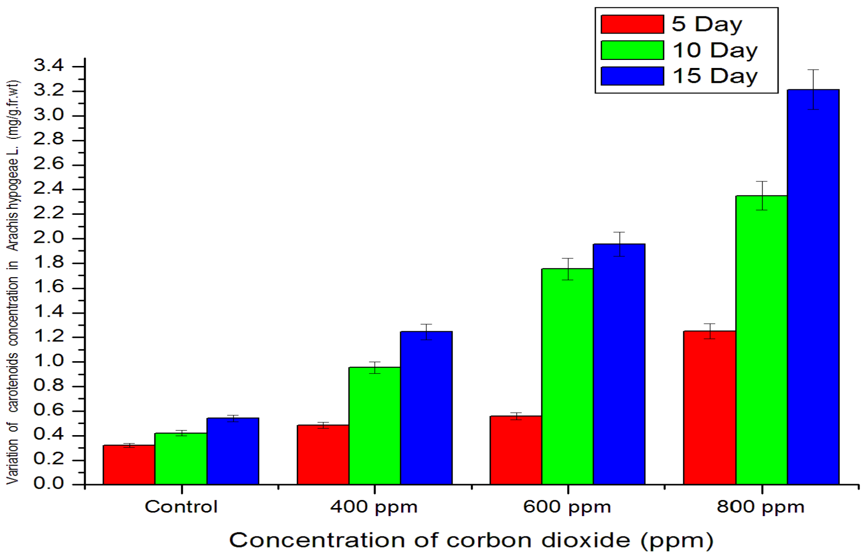

Figure 1: Impact of CO_2 concentration of carotenoids in *Arachis hypogaea*L. (mg/g.fr.).

Table 2: Impact of CO_2 interaction on proline content in*Arachis hypogaea*L. (mg/g.fr.wt).

Name of the plant	Concentration of CO_2	5^{th} day	10^{th} day	15^{th} day
*Arachis hypogaea*L.	Control	0.541±0.027	0.648±0.324	0.754±0.037
	400 ppm	0.7511±0.375	0.8541±0.0427	0.9451±0.0472
	600 ppm	0.9487±0.473	1.214±0.060	1.854±0.0927
	800 ppm	1.555±0.077	2.114±0.105	2.541±0.1270

Values represented in table are means of three replicates ± SE (Standard Error)
Values in the column bearing one or more than one common letters in superscript are not significantly different from each other ($P \leq 0.05$) according to DMR (Duncan Multiple Range) test.

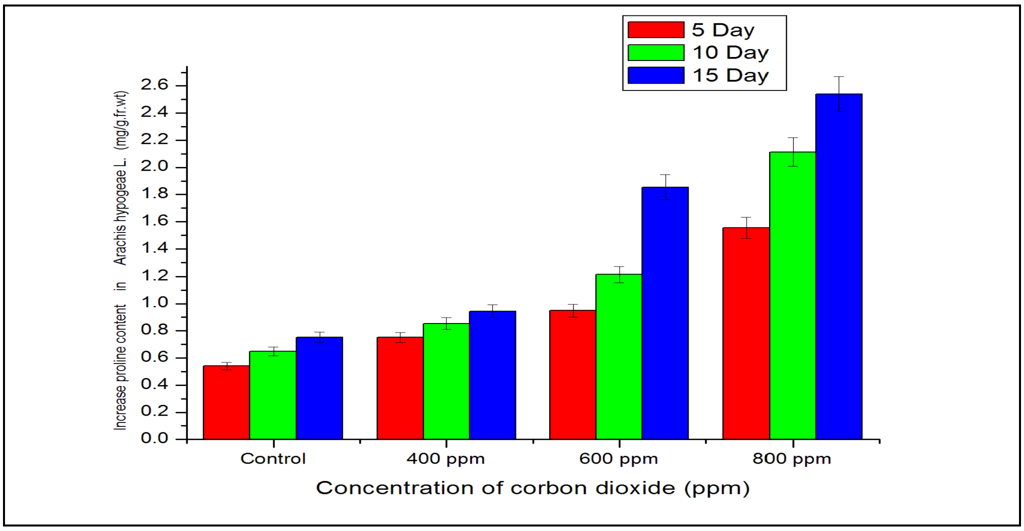

Figure 2: Impact of CO_2 concentration of proline in *Arachis hypogaea*L. (mg/g.fr.wt).

Estimation of total carotenoids
Total carotenoids were determined as per the method of Jensen and Jensen (1971).

Extraction
1g (one gram) of freshly harvested leaf material made into small pieces and macerated with acetone in a mortar. The extract was centrifuged and residue thus obtained was again restricted followed by centrifugation. The procedure was repeated until no more pigments were extracted.

The supernatants were pooled up and the acetone was removed in vacuo**.** The residue after complete removal of acetone was dissolved in a small volume of 10% (UV) methanolic KOH solution (10 ml final solution for each mg of pigment).

The mixture was allowed to stand at room temperature for two hours and then transferred to a separating funnel. The reaction was stopped by the addition of 5% (W/V) aqueous XaCl solution. After separation, the aqueous phase was extracted thrice with petroleum ether and the combined petroleum to remove methanol. The presence of salt facilitated the separation of phases and prevents the formation of emulsions. The ether extract was dried over anhydrous sodium sulphate and the petroleum ether was removed in *vacuo*.

Estimation of Proline
Proline was estimated according to the procedure of Bates et al., (1973). One gram of freshly harvested leaves were taken and washed thoroughly with tap and distilled water. leaves were blotted dry and cut into small bits. leaf bits were ground in 10 ml of 3% aqueous sulfosalicylic acid and filtered through Whatman No.l filter paper. for two ml of filtrate 2 ml of acid ninhydrin reagent (1.25g ninhydrin, 20 ml of glacial acetic acid, 20 ml of 6M Phosphoric acid) and 2 ml of glacial acetic acid were added and samples were kept in boiling water bath for one hour. after one hour of heating the samples were kept in an ice bath to terminate the reaction. Four ml of toluene was added to each tube and mixed vigorously. The chromophore was aspirated from the aqueous phase and warmed the samples to room temperature. The samples were read at 520 nm in schimadzu UV-VIS Spectrophotometer. Proline concentration in the samples was computed based on a similarly prepared standard curve for proline.

Impact of CO2 **concentration on total careteniods of *Arachis hypogaea* (L.),**
Elevated CO2 conditions alter the plant biochemical parameters of Carotenoids fractions were estimated in leaves of control and treated plants from 5^{th}, 10^{th} and 15^{th} day after treatment at 5 days interval in *Arachis hypogaea* (L.), among control plants the response was positive in elevated CO2 possessed maximum level of total careteniods in *Arachis hypogaea* (L.), the response

was positive for total caroteniods content under elevated CO_2. the data on plant *Arachis hypogaea* (L.), for caroteniods content presented in **(Figure 1)**, indicated significant differences in elevated CO2of 400 ppm, 600 ppm and 800 ppm in 5th, 10th and 15thdays compared with ambient CO2 in all stages. The carotenoid content increased continuously from germination to 15th days. On the basis of this investigation, the maximum caroteniods content of *Arachis hypogaea* (L.) was recorded at 15th day at 800 ppm (3.214 mg/gft) is given in **(Table 1)** as per junkys at al.,2012. elevated CO2 almost did not affect the concentration of carotenoids in the leaves of investigated species (barely, tomato, fat-hen), however spastically significant increase in the concentration of carotenoids was registered in the case of pea and beetroot at 500 ppm and in the case of soybean at 700 ppm of CO2 .

Impact of CO_2 concentration on total proline of *Arachis hypogaea* L

Elevated CO2 conditions triggers a wide range of physiological and biochemical processes tend to alter the foliar chemistry of plants, i.e. proline contents of leaves. Proline fractions were estimated in leaves of control and treated plants from 5th , 10th and 15th day after treatment at 5 days interval in *Arachis hypogaea* (L.), Among control plants the response was positive in elevated CO2 possessed maximum level of proline in *Arachis hypogaea* (L.), The data on plant *Arachis hypogaea* (L) total proline content presented in **(Figure 2)**, indicated significant differences in elevated CO2 of 400 ppm, 600 ppm and 800 ppm in 5th, 10th and 15th compared with ambient CO_2 in all stages. The proline content increased continuously from germination to 15th days. On the basis of this investigation, the maximum total proline of *Arachis hypogaea* (L)., was recorded at 15th day at 800 ppm (2.541 mg/gft) is given in **(Table 2)**. The concentration of proline in *Arachis hypogaea* (L.) of ambient and different elevated CO_2 ppm over days are shown in **(Figure 2)**. Proline is one of the osmolytes, which increase faster than other amino acids in plants under water deficit stress and help the plants to maintain cell turgor (Zhao *et al.*, 2008). under water deficit environment, drought stress increased proline content, this increasing roles as an osmotic compatible and adjust osmotic potential, which resulted in drought stress avoidance in chickpea (Mafakheri *et al.*, 2011), proline accumulation can be used as a criterion for drought resistance assessment of varieties (Vijayalakshmi *et al.*, 2010; Gunes *et al.*, 2008).

CONCLUSION

We conclude that, the elevated CO_2 conditions significantly increased the carotinoid and proline contents of arachis hypogaea. Increased carotenoid content indicate that high-elevated CO_2 has positive impact on arachis hypogaea, and positive enhancement on drought resistant amino acid proline content of arachis hypogaea. The variability in response to elevated CO_2 is useful in selecting genotype or developing new varieties in order to fit them into the predicted future climatic conditions.

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