



Impact of High Elevated CO₂ on Growth and Total Leaf Soluble Protein Content in *Arachis Hypogaea* (L)

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Abstract

Increasing atmospheric CO₂ concentration has led to concerns about global changes to the environment. One area of global change that has not been fully addressed is the effect of elevated atmospheric CO₂ on agriculture production inputs. Groundnut is a very important edible oilseed crop raised mostly under rainfed situations worldwide. This experiment was conducted to evaluate the effects of normal CO₂ level and elevated concentration by the raised in open top chamber with different concentrations of CO₂ levels (200ppm, 400ppm, 600ppm,) on *Arachis hypogaea* (L) to investigate the various physiological growth changes (growth of root, shoot length) and total leaf soluble protein content was measured at days of 5th, 10th, and 15th intervals. The results showed that in *Arachis hypogaea* (L) the CO₂ elevation may enhance higher root, shoot and leaf soluble protein content was significantly increased with increase in concentration of the CO₂.

Keywords: Elevated CO₂, Root & shoot growth, leaf soluble protein,.

Introduction

Carbon dioxide (CO₂) is the primary greenhouse gas emitted through anthropogenic activities Atmospheric CO₂ concentration has increased from 280 ppm to 390 ppm from the preindustrial revolution to the present and at the end of the 21st century it may reach even up to 750 to 1000 ppm [1]. Carbon dioxide is naturally present in the atmosphere as part of the Earth's carbon cycle, enhanced atmospheric CO₂ concentration influences the functions of ecosystem including agricultural yield. It is well established that CO₂ is a plant nutrient and its enrichment is expected to enhance crop growth and productivity [2] by increasing photosynthesis and water use efficiency and by decreasing transpiration through reduced stomatal conductance. This is because the different mechanism of carbon fixation by the two types of photosynthesis, such increase occurs due to a gain in carboxylation at the expense of oxygenation reaction until ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO) reaches saturation.[3]. This increase is likely to affect biota indirectly via climate change, and directly by producing changes not only in plant growth and allocation, but also in plant tissue chemical composition. Legumes, being more responsive to elevated CO₂ than other plants, will have a competitive advantage [4].

Peanut (*Arachis hypogaea* L.), also known as groundnut, earthnut, and ground bean, is the world's fourth most important source of edible vegetable oil, and third most important source of vegetable protein. Peanut was grown on 23.91 million hectares worldwide, with a total production of 36.60 million tons, and an average yield of 1531 kg/hectare in 2009 (<http://faostat.fao.org>). China, India, Nigeria, the United States of America, 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>). Peanut has traditionally been used as a source of oil; however, its worldwide annual protein harvest has reached nearly 4.5 million tons. Crude protein content of whole seed peanuts is estimated to be around 25%, followed by carbohydrates (16%), and monosaturated fats (24%). Many plant species respond to enriched atmospheric CO₂ by enhanced photosynthetic rates and increase in biomass, as well as alterations in leaf quality factors. At present there is an urgent need to understand the existence of response variability in available germplasm to fine tune the assessment of their impacts. Significant intra-specific variation in yield response to elevated CO₂ was reported in crops such as rice [5], cowpea [6], wheat [7], soybean [8], common bean [9], blackgram [10], and also in several non-crop species [11].

Legumes, being more responsive to elevated CO₂ than other plants, will have a competitive advantage [4], when grown under elevated CO₂. Within legume species, nodulating genotypes, or N fixers, are more responsive to elevated CO₂ than non-fixers [12]. It provides dietary protein and high-grade fat to low income as well as vegetarians. India is the major groundnut producing country in the world and stands third in exporting of this commodity [13]. Since legumes can supply nitrogen via symbiotic nitrogen fixation, both seed and forage legume crops are expected to respond relatively more to a rise in CO₂ concentration. Groundnut being a C₃ legume crop, elevated CO₂ in the atmosphere is expected to affect this crop in terms of its growth, physiological and yield parameters. Increase in photosynthetic rate, biomass and pod yield of groundnut due to elevated CO₂ in certain virginia groundnut genotypes was reported [14]. In this context, the present work aims at evaluating the variability in growth and physiological parameters of *Arachis hypogaea* (L) when exposed to elevate different of 200ppm, 400ppm, 600ppm CO₂ concentration.

Material and Methods

Seed materials:

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

Experimental conditions and CO₂ exposure:

The study was conducted in pots (33 cm diameter × 23 cm depth) at the controlled CO₂ chambers, in SV University, Tiruparti, A.P, India. (Each chamber was 2.4 m in diameter 2.5 m in height and was closed at the top). The experiment was organized in a randomized complete block design (RCBD) with four replications. 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 CO₂ for seven days and then transferred to the pots. Plants were maintained in a controlled-climate growth and photoperiod for a week to allow plant acclimation to the growth chamber conditions prior to exposing plants to CO₂ treatments. The CO₂ concentration was manipulated automatically by controlling the amount of CO₂ gas injected, as described previously (Lee *et al.*, 2000). Twenty days old seedlings were progressively exposed to 200 ppm, 400 ppm and 600 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). Then the roots, shoot were harvested for morpho-biochemical characters and leaves for protein extraction. After sampling the leaves were immediately frozen in liquid at -80 C until nitrogen stored until required. The experiment was repeated in five replicates.

Plant growth measurements:

The data on growth parameters were recorded at i.e. 5, 10 and 15 days after sowing respectively in three replications. The plant samples in triplicate at two growth stages were collected from both ambient and elevated CO₂ conditions by uprooting them carefully and their roots were washed gently and separated into roots, stems and leaves. The leaf area was measured with photo-electronic leaf area meter (LI-3100, LI-COR). Plant root & shoot length were measured, and leaf were recorded.

Total leaf soluble protein estimations:

Leaves of control and treated plants were used for the estimation of total protein content was estimated by Lowry *et al.*, 1951 by using Folin – Ciocalteu reagent. Extraction is usually carried out with buffers used for the enzyme assay. Weigh 500 mg of the sample and grind well with a pestle and mortar in 5-10 ml of the buffer. Centrifuge and use the supernatant for protein estimation, pipette out 0.2 to 1 ml of working standard in to series of test tubes. Pipette out 0.1 ml and 0.2 ml of the sample extract in two other test tubes, makeup the volume to 1 ml in all the test tubes, distilled water serve as the blank. Add 5 ml alkaline copper reagent to each tube including the blank, mix well and allow standing for 10 min., then add 0.5 ml of Folin –Ciocalteu reagent incubate room temperature in dark for 30 min. Blue colour is developed and read at 660 nm by spectrophotometer.

Results and Discussion

Carbon dioxide (CO₂) is essential for photosynthetic carbon reduction. Rising CO₂ concentration in the atmosphere can have both positive and negative consequences on plant growth. Elevated CO₂ tend to increase size and biomass of plant component in most C₃ plants. The accumulated extra photo assimilates during vegetative development mainly partitioned into structural components such as stem and petioles which in turn support the leaves for better photo assimilation. Hence, differential response of genotypes of a particular crop to elevated CO₂ is a result of change in allocation of photo assimilates. In the present study, the groundnut cultivars were observed to possess higher values for most of the yield parameters studied at elevated levels of CO₂ 200 ppm, 400 ppm and 600 ppm when compared with ambient control, the root & shoot growth and soluble protein parameters of selected for groundnut showed positive response to elevated CO₂ at two growth stages. The mean performance for growth and physiological parameters were presented in Tables 1-3.

Shoot, Root and total protein Growth:-

Absorption of Carbon dioxide in plants help to increase in plant growth as well as roots grow rapidly during germination and seedling stages and within 5 or 6 days after sowing, the taproot may grow 10–16 cm deep and develop a number of lateral roots. Root and shoot growth of reated *arachis hypozea*. L plants were higher than the control plant at 200 ppm 2.3 cm, at 400 ppm 3.5 cm, at 600 ppm 4.6 cm. Compare to control, treated plant at 200 ppm the root length increased at 5th day 2.3 cm, 10th day 4.4 cm and 15th day 4.6 cm, at 400 ppm the root length increased at 5th day 3.4 cm, 10th day 4.9 cm and 15th day 5.1 cm and at 600 ppm the root length increased at 5th day 5.1 cm, 10th day 5.8 cm and 15th day 6.7 cm. (Figure - 1 & Table-1). Ketring and Reid (1993) found that root length density significantly increased at 10 cm depth until 80 days. At 40–45 days, roots had penetrated to a depth of 120 cm and spread laterally at least 46 cm. The investigation of Gregory and Reddy (1982) indicated that the total root length of cultivar. In peanut, it has been recently reported that a doubling of ambient [CO₂] enhances leaf photosynthesis by 27% and seed yield by 30% across a range of daytime growth temperatures from 32 to 44 °C [2].

Shoot growth of reated *arachis hypozea*. L plants were higher than the control plant at 200 ppm 4.2 cm, at 400 ppm 5.7 cm, at 600 ppm 6.6 cm. compare to control, treated plant shoot growth of reated *Arachis hypozea*. L plants were higher than the control plant. At 200 ppm the shoot length increased at 5th day 4.2 cm, 10th day 5.7 cm and 15th day 6.6 cm, at 400 ppm the shoot length increased at 5th day 5.1 cm, 10th day 6.2 cm and 15th day 7.2 cm and at 600 ppm the shoot length increased at 5th day 7.4 cm, 10th day 8.3 cm and 15th day 9.7 cm.(Figure - 2 & Table-2). The above shoot responses of groundnut with elevated CO₂ were in tune with previous reports on performance of different crops. Plant height increased in black gram from 9 to 34% with 700ppm, 2 to 21% with 550 ppm [18] at different growth stages. Elevated CO₂ resulted greater leaf area, root length, and plant height along with improved dry matter production in mung bean at different growth stages [19]. Leaf area and stem length increased with elevated CO₂ in Tainan 9 groundnut variety at 25/15 °C [20].

As compared to ambient condition, majority of biomass parameters improved at 6000 ppm CO₂. The improvement of leaf and stem biomass of ground nut was 20%, root biomass of 13% and total biomass of 19%. The enhanced leaf,

stem and root biomass resulted overall increase in total biomass of treated ground nut plant. Highly significant improvement of leaf biomass with elevated CO₂ was observed in Similar results were reported in different pulse and oil seed crops. With an increase in CO₂ concentration, leaf dry weight of black gram increased up to 71% and groundnut by 17% [19]. The variation in the responses of groundnut genotypes to elevated CO₂ was reported where in higher response was in TPT-1 for stem dry weight (53%), and TPT- 4 for root dry weight (79%) [18]. Increase in CO₂ level from 400 to 800 $\mu\text{mol mol}^{-1}$ increased above ground biomass in Tainan 9 groundnut variety at 25/15 °C [20] and total biomass increased at 600 $\mu\text{mol mol}^{-1}$ CO₂ in *Vigna radiata* L. [19]. Based on field studies, Hardy [21] reported that higher dry matter and vegetative growth of groundnut at 1000 to 1500 ppm CO₂ and it was attributed to decreased photorespiration, delayed senescence and increased plant density.

Total soluble protein estimation:

The effect of elevated CO₂ on leaf soluble protein content in ground nut leaves was significant (Figure -3 & Table 3). The concentration of soluble protein in ground nut leaves protein increased at higher elevated CO₂ level. Soluble protein content was increased *arachis hypogaea*. L plants were higher than the control plant at 200 ppm 11.225 mg/gft, at 400 ppm 12.458 mg/gft, at 600 ppm 13.274 mg/gft, compare to control, treated plant protein content of *arachis hypogaea*. L plants were higher than the control plant. At 200 ppm the protein content increased at 5th day 13.242 mg/gft, 10th day 14.578 mg/gft, and 15th day 15.211 mg/gft. At 400 ppm the protein content increased at 5th day 14.211 mg/gft, 10th day 15.985 mg/gft and 15th day 16.237 mg/gft and at 600 ppm the protein content increased at 5th day 15.484 mg/gft, 10th day 16.585 mg/gft, and 15th day 17.325 mg/gft. Though genotypic difference was observed for influence of enhanced CO₂ on gs, all genotypes recorded reduced transpiration rate. Lewis [28] recorded a reduction of 12% in transpiration rate of Douglas fir seedlings at 540 ppm, whereas Jianlin et al. [23] also reported reduction of transpiration in crop plants such as peanut (24%) maize (84%) sorghum (73%), millets (75%), soybean (29%), sweet potato (45%), rice (50%) at elevated CO₂. With growing population, degrading natural resources and changing food habits more pressure is on improvement of agricultural productivity in general and oilseed production in particular. Groundnut (*Arachis hypogaea* L.) is an important edible oil seed crop with 44-56% oil and 22-30% protein [13]. It provides dietary protein and high-grade fat to low income as well as vegetarians. India is the major groundnut producing country in the world and stands third in exporting of this commodity [14]. In the present investigation, significantly higher quantity of linoleic acid and oleic acid was found in the seeds of CO₂ enriched plants. The increase in oleic acid content due to elevated CO₂ is a positive effect, as it would provide better thermo-stability to the edible oil which is desirable from the cooking and nutritional point of view. Protein content is an important indicator of physiological status of plants.

Conclusion

Enhanced CO₂ condition improved all the growth and biomass parameters in the selected five groundnuts due to improved photosynthesis. This clearly reveals that the mechanism of enhanced CO₂ action. It was also evident that root, shoot and leaf characters of recorded response with elevated CO₂. Since there could be various abiotic stresses faced by plant at the same point of time, the extent of the interaction between elevated CO₂ and other parameters can vary, depending on the species and genotype, gas concentrations, and other experimental and environmental conditions. The variability in response to elevated CO₂ is useful in selecting genotype or developing new varieties in order to fit them into the predicted future climatic conditions.

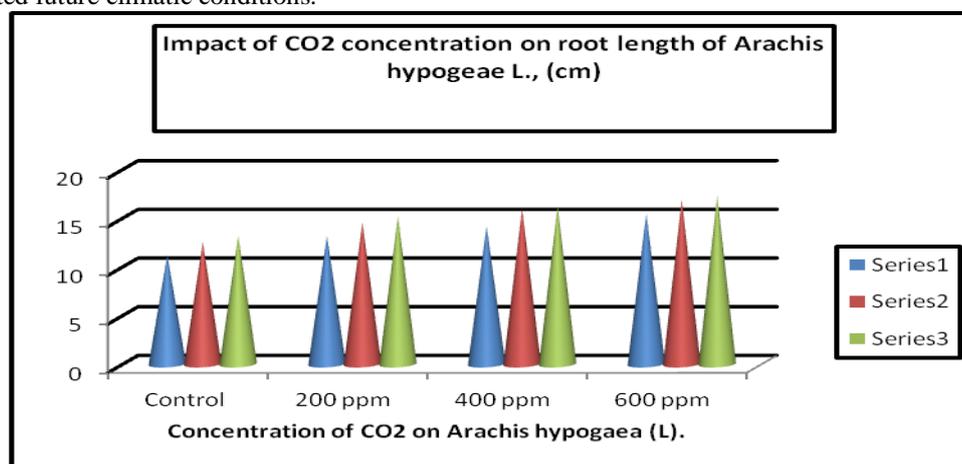


Figure 1: Increase (%) of root parameters at elevated (200 ppm, 400 ppm, 600 ppm) over ambient CO₂ of *Arachis hypogaea* L.,

Table 1: Increase (%) of root parameters at elevated (200 ppm, 400 ppm, 600 ppm) over ambient CO₂ of *Arachis hypogaea* L.,

Name of the plant	Concentration of CO ₂	5th day	10th day	15th day
<i>Arachis hypogaea</i> L.	Control	2.3	3.5	4.6
	400 ppm	3.4	4.4	5.1
	600 ppm	4.1	4.9	5.7
	800 ppm	5.1	5.8	6.7

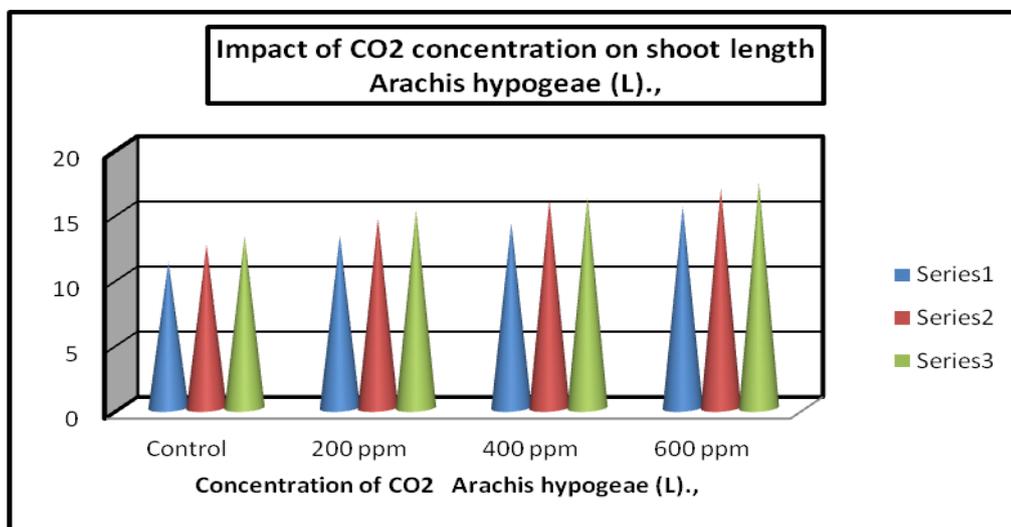


Figure 2: Increase (%) of shoot parameters at elevated (200 ppm, 400 ppm, 600 ppm) over ambient CO₂ of *Arachis hypogaeae* L.,

Table 2: Increase (%) of root parameters at elevated (200 ppm, 400 ppm, 600 ppm) over ambient CO₂ of *Arachis hypogaeae* L.,

Name of the plant	Concentration of CO ₂	5th day	10th day	15th day
<i>Arachis hypogaeae</i> L.	Control	4.2	5.7	6.6
	400 ppm	5.1	6.2	7.2
	600 ppm	6.4	7.5	8.4
	800 ppm	7.4	8.3	9.7

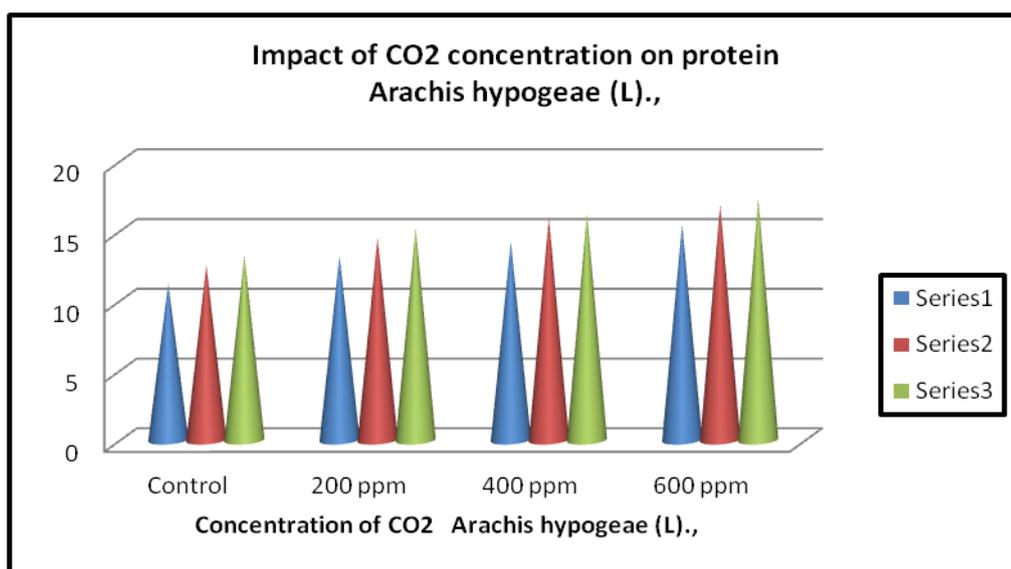


Figure 3: Increase (%) of soluble protein parameters at elevated (200 ppm, 400 ppm, 600 ppm) over ambient CO₂ of *Arachis hypogaeae* L.,

Table 3: Increase (%) of soluble protein parameters at elevated (200 ppm, 400 ppm, 600 ppm) over ambient CO₂ of *Arachis hypogaeae* L.,

Name of the plant	Concentration of CO ₂	5th day	10th day	15th day
<i>Arachis hypogaeae</i> L.	Control	11.215	12.548	13.214
	400 ppm	13.222	14.558	15.211
	600 ppm	14.211	15.985	16.231
	800 ppm	15.484	16.885	17.325

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