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GROWTH AND YIELD OF CHICKPEA UNDER ELEVATED CARBON DIOXIDE CONCENTRATION

MADAN PAL*, S. TALAWAR, P.S. DESHMUKH, C. VISHWANATHAN, S. KHETARPAL, PRAMOD KUMAR AND DEVANAND LUTHRIA[1]

Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi-110 012
[1]United States Departemnt of Agriculture, Beltsville, Maryland 20705

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SUMMARY

Chickpea (*Cicer arietinum* L.) cultivar Pusa 1108 was grown inside open top chambers (OTCs) and exposed to ambient (CA, 370±20 µl l^{-1}) and elevated (CE, 550±50 µl l^{-1}) CO_2 from germination to maturity of the crop to determine its growth and yield response. The plants exposed to elevated CO_2 showed increase in growth characteristics, viz. shoot length, total number of branches and leaf area per plant. Significant increase in leaf and shoot dry weight was recorded in elevated CO_2 grown plants. The concentration of non structural carbohydrates such as sugars and starch in leaves was higher under elevated CO_2 grown plants, which indicated higher photosynthetic activity. Total carbon concentration increased but the nitrogen concentration decreased in the leaves and resulted in higher C/N ratio. The seed yield of elevated CO_2 grown plants was higher due to significant increase in number of seeds per plant. This study suggests that rising atmospheric CO_2 in future may increase dry matter production and yield in chickpea but reduction in nitrogen concentration may alter their protein levels.

***Key words: Cicer arietinum*, elevated CO_2, growth, nitrogen, sucrose phosphate synthase, yield**

INTRODUCTION

During the past few decades changes in the earth climate have become the focus of scientific and social attention. One of the pre-eminent and incontrovertible manifestations of climate change is the increase in atmospheric CO_2 concentration, from approximately 280 to 379 µl l^{-1} (IPCC 2007). During the last twelve years the rate of increase of CO_2 is 1.9 µl l^{-1} per year and is expected to be as high as 570 µl l^{-1} by the middle of this century. A predicted consequence of this rise in CO_2 and other greenhouse gases is the warmer temperature of the earth surface. The Intergovernmental Panel on Climate Change (IPCC) has projected a 2.0 to 4.5°C increase in the global average temperature of the earth surface by the end of this century (IPCC 2007). Both CO_2 and temperature are the key variables of global climate and may cause significant changes in crop productivity through their direct or indirect effects on crops, soils, livestock pests, etc. The increase in atmospheric CO_2 may have fertilization effects for many plants especially C_3 crop species which constitute more than 90% of terrestrial species. On the other hand, a rise in atmospheric temperature may affect plant processes differently, like increasing the rate of respiration and evapotranspiration, a reduction in crop duration, or altering the partitioning of photoassimilates and thus may have tremendous impact on crop productivity (Aggrawal and Sinha 1993).

Numerous studies report enhanced rates of photosynthesis and plant growth in C_3 crop species when

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*Corresponding author, E-mail: madanpal@yahoo.com grown under elevated levels of CO_2 concentration. It has been well documented that the photosynthetic process is limited by the current level of atmospheric CO_2 in C_3 species. A higher level of CO_2 concentration in the atmosphere reduces this limitation and enhances the rate of photosynthetic CO_2 assimilation (Drake *et al.* 1997 and Ghildiyal and Sharma-Natu 2000). Higher concentration of CO_2 in the atmosphere decreases the rate of photorespiration as it competes with O_2 and suppresses the oxygenation reaction of Rubisco. This effect of elevated CO_2 is of great importance as it results in increased net photosynthesis without requiring additional light, water, nitrogen or nutrients (Long 1991). C_4 plants show negligible effect in response to elevated CO_2 exposure as their carbon assimilation cycle acts as a CO_2 concentrating mechanism and provides a high CO_2 concentration (Bowes 1993).

Chickpea (*Cicer arietinum* L.) is an important legume crop which has been grown in semi-arid region of the world for centuries. It is primarily cultivated in the Indian subcontinent, the Middle East, near the Mediterranean, and North Africa. Chickpea is the third most important pulse crop in the world after dry beans and dry peas. In India, chickpea is either consumed as whole, shelled, dhal (split chickpea), or as besan (dhal flour) that is used in the preparation of a large number of snacks. The per capita consumption of chickpea in India is around 5.37 kg per year (Yadav *et al.* 2007). There have been a large number of publications focusing on the influence of elevated CO_2 concentration on the growth of several cereal (rice, wheat, maize) and pulse (soybean, mungbean) crops. However, information on the influence of elevated CO_2 concentration on growth of chickpea in India is rather meagre. In addition, chickpeas are capable of fixing N_2 symbiotically and also have great sink potential owing to its indeterminate growth habit. Hence, the present research study was planned to investigate the growth and photosynthetic response of chickpea to elevated CO_2 concentration.

MATERIALS AND METHODS

Elevated CO_2 exposure in open top chambers: The chickpea plants [(*Cicer arietinum* L.) cv. Pusa 1108] were raised in earthen pots at the Indian Agricultural Research Institute, Division of Plant Physiology, New Delhi, India during the *rabi* season from November 25, 2005 to April 8, 2006. The seeds were inoculated with fresh inoculum of *Mesorhizobium ciceri SP4* @ 8.0 g kg^{-1} and then sown in 3.4 dm^3 earthen pots containing sterilized sandy loam (3:1) soil. One week after germination, the plants were thinned to two plants per pot in order to get uniform growth of the seedlings. Recommended cultural practices were followed. In the experimental set up, four modified naturally lit open top chambers (OTCs) as described by Rogers *et al.* (1983) were used to study the response of the chickpea crop to elevated CO_2. Each OTC was lined with PVC (poly vinyl chloride) sheets transmitting more than 85% of incident solar radiation. The height and diameter of the OTC was 2.5 m and 2.0 m, respectively. The base of the chamber was made with a hollow circular PVC ring having perforations 30 cm apart for passing the CO_2-air mixture into the chamber. Pure CO_2 gas (99.7% v/v CO_2 with less than 10 ppm CO) was dispersed under controlled pressure (through a pressure gauge) and mixed with air. The CO_2-air mixture was forced into the chamber through a blower. Pure CO_2 gas used for the study was purchased from M/s Sigma Gas Service, New Delhi. The desired concentration of CO_2 (550 $\mu l\ l^{-1}$) was achieved with the help of gas flow-meter and gas regulator. In control chambers, only ambient air was supplied through blowers to maintain similar environmental condition, except for the CO_2 level. Monitoring of the micro-atmospheric CO_2 concentration was done daily throughout the crop-growing season using a portable infrared gas analyzer (IRGA, Model LI-6200, LI COR, Lincoln, Nebraska, USA). One set of plants were grown under ambient CO_2 (370 $\mu l\ l^{-1}$) and another set of plants were grown under elevated CO_2 (550 $\mu l\ l^{-1}$) in the OTCs. CO_2 exposure was carried out daily during daylight hours immediately after emergence of seedlings and continued throughout the growth period until the crop matured. Growth and biochemical observations were recorded through destructive sampling at three stages: 30 (vegetative), 65 (pre-flowering), and 90 (post-flowering stage) days after initiating CO_2 exposure (DAE). These durations correspond to vegetative, flowering, and pod development stages of chickpea.

Growth and yield parameters: For measurement of plant growth parameters, plants were separated into stem and leaves and their leaf area was determined using a

Li-COR Leaf Area Meter (Model LI-3100). For dry weight determination, plant samples were dried in a hot air oven at 70°C until constant weights were obtained and the dry weight was recorded using an electronic balance (1212MP, Sartorius, GMBH Gottingen, Germany). Yield parameters were recorded after harvesting the crop.

Sugar and starch estimations: Sugars were extracted from leaf sample (1.0 g fw) by boiling in 80 % (v/v) ethanol in water and clarified following the method of McCready *et al.* (1950). The aliquot of clarified sugar extract was used for the determination of sugar content using Nelson's arsenomolybdate method (Nelson 1944). The concentration of total sugars was expressed as mg glucose g^{-1} leaf dry weight. The dry weight of leaf residue left after sugar extraction was determined and used for starch estimation. The dried and powdered leaf residue (0.05 g) was hydrolyzed in 1N HCl in a glycerine bath at 112-115 ^{0}C for determination of starch content. The samples were washed repeatedly with distilled water until a negative test was obtained with iodine. Starch content was determined by the anthrone method (McCready *et al.* 1950) and results were expressed as mg glucose g^{-1} leaf dry weight.

Sucrose content and sucrose phosphate synthase activity: The sucrose (sucrose phosphate) content of the samples was determined by the anthrone method of Van Handel (1968). Five ml of anthrone reagent and 30 ml of H_2O were added to the sample and incubated at 40^{0}C in a hot water bath for 15 min and the absorbance was recorded at 620 nm and sucrose content was expressed as mg g^{-1} fw. The sucrose phosphate synthase (SPS) enzyme was extracted following modified method of Huber and Bickett (1984). Leaf tissue (1 g fw) was ground in a precooled mortar and pestle with 8 ml of grinding medium containing 50 mM Hepes-NaOH (pH 7.5), 5 mM $MgCl_2$, 1 mM EDTA, 2.5 mM DTT, and 0.5% BSA. The extract was centrifuged at 38,000 g for 10 min at 0°C. SPS was assayed following Rufty and Huber (1983) by measuring fructose 6-phosphate dependent formation of sucrose (+ sucrose-P) from UDP glucose. The assays were initiated by addition of 20 µl of enzyme to reaction mixtures containing 50 mM Hepes-NaOH (pH 7.5), 5 mM $MgCl_2$, 7.5 mM fructose 6-P in a total volume of 0.1 ml. The reactions were performed in test tubes at 30°C using a shaking water bath and terminated after 10 minutes by adding 0.1 ml of 30% KOH. Background was determined by including zero reaction time samples. SPS activity was expressed as µg g^{-1} fw h^{-1}.

C and N estimations: Total nitrogen content of plant samples was estimated following the Kjeldahl method (Jackson 1973). Dry leaf samples (1 g) harvested at the three different stages of growth as described above were subjected to digestion and distillation. N concentration was expressed as per cent of leaf dry weight. Estimation of carbon concentration was determined following the wet digestion modified method of Walkley and Black (1934). The dried sample (0.05 g) was oxidized with a mixture of potassium dichromate and concentrated sulphuric acid using the heat of dilution of acid. The unused potassium dichromate was estimated by back titration with ferrous ammonium sulphate. The carbon concentration was expressed as per cent of leaf dry weight.

Statistical analysis: The experiment was conducted in a completely randomized block design (CRD) with three replications for each control and treatment. Statistical analysis of the data was done by analysis of variance (ANOVA) as given by Panse and Sukhatme (1967) and the critical difference (CD) values were calculated at the 5% probability level.

RESULTS AND DISCUSSION

Exposure to elevated CO_2 in open top chambers increased the growth of chickpea plants. Shoot length and leaf area per plant increased significantly in the elevated CO_2 grown plants. The extent of stimulation in plant growth due to elevated CO_2 exposure was quite similar for all the growth parameters. The average increase in shoot length and leaf area was around 30 % in plants exposed to elevated CO_2 (Table 1). The effect of elevated CO_2 was evident in chickpea plants from 30 days after exposure (DAE) till crop maturity. The increase in leaf area per plant was due to production of more branches (15%) in the elevated CO_2 grown plants. The increase in shoot length and leaf area was accompanied by higher leaf and shoot dry weight in elevated CO_2 grown chickpea plants, which increased

Table 1. Plant growth parameters of chickpea grown at ambient (CA, 370±20 µl l^{-1}) and elevated CO_2 (CE, 550±50 µl l^{-1}) concentration.

Days after initiating exposure (DAE)	CO_2 concentration	Shoot length (cm $plant^{-1}$)	Number of branches ($plant^{-1}$)	Leaf area (cm^2 $plant^{-1}$)	Leaf dry weight (g $plant^{-1}$)	Shoot dry weight (g $plant^{-1}$)
30	CA	12.45	4.1	66.91	0.65	1.40
	CE	15.67 (+26)	4.6 (+12)	89.45* (+34)	0.81* (+25)	1.83* (+31)
65	CA	21.3	6.1	150.24	1.22	2.73
	CE	28.7* (+35)	7.0 (+15)	197.15* (+31)	1.70* (+39)	3.77* (+38)
95	CA	31.0	6.5	276.67	1.78	4.67
	CE	40.6* (+31)	7.6 (+17)	358.5* (+30)	2.43* (+37)	6.70* (+43)

Values in parentheses indicate per cent change due to elevated CO_2 exposure, *difference between CA and CE significant at $p<0.05$.

by an average of 33 and 37%, respectively. The increase in leaf dry weight (25%) was less at 30 DAE compared to later stages of growth. Similarly shoot dry weight increased between 31 and 43% throughout the growth period. Similar growth response to elevated CO_2 has been reported in other pulse crops like soybean (Allen *et al.* 1991), dry bean (Prasad *et al.* 2002), mungbean (Srivastava *et al.* 2001) and cowpea (Ellis *et al.* 1995). In soybean exposure to elevated CO_2 caused increases in main stem height, branch length, stem diameter, individual leaf, and leaf area per plant. Increase in growth under elevated CO_2 has been attributed to availability and partitioning of more assimilates to different growing parts of the plants (Prasad *et al.* 2005). Elevated CO_2 exposure in soybean increased the number of branches per plant and provided more sites for leaf formation (Rogers *et al.* 1986).

Carbohydrates, nitrogen and carbon concentration and C:N ratio: The concentrations of both sugars and starch were higher in chickpea plants exposed to the elevated CO_2 concentration. This could be attributed to increased photosynthetic rate under higher levels of atmospheric CO_2. The starch concentration of plants grown under elevated CO_2 was significantly higher at all the stages than that seen with the soluble sugars. The average increase in starch and sugar concentration was 35 and 23 percent, respectively in the chickpea plants grown under elevated CO_2 conditions (Table 2). The magnitude of the increase in starch concentration was similar at all the stages. On the other hand, level of sugars varied between 15 and 32% at different stages of growth. The maximum increase of 32% in sugar concentration was observed 65 DAE. Higher concentration of sugars in the leaves, however, causes decrease in Rubisco SSU gene expression, consequently, down regulates photosynthesis (Sharma-Natu and Ghilidiyal 1993, Pandurangam *et al.* 2006). Similar changes in carbohydrate concentration have been reported in other legume crops grown under higher levels of CO_2 (Allen *et al.* 1998). The accumulation of starch and better yield response has also been reported in mungbean and soybean grown under high atmospheric CO_2 concentration (Vu *et al.* 1989, Ghildiyal *et al.* 1998, Sharma-Natu *et al.* 2004). Starch accumulated in the leaves mobilized during the night becomes additional sink for photoassimilates during the day. Some reports suggest that plants having substantial sink capacity show better response to elevated CO_2 exposure as compared to those with smaller sink capacity (Bowes 1993, Sharma-Natu *et al.* 1997, 2004). This suggest that chickpea plants can take better advantage of rising atmospheric CO_2 in future in maintaining higher photosynthesis as they accumulate less sugars compared to starch under elevated CO_2. A similar increase in soluble sugars and starch has been shown in *Phaseolus*

Table 2. Total sugars, starch, nitrogen, carbon concentration and C/N ratio in chickpea plants grown at ambient (CA, 370±20 µl l^{-1}) and elevated CO_2 (CE, 550±50 µl l^{-1}) concentration.

Days after initiating exposure (DAE)	CO_2 concentration	Total sugars (mg g^{-1} dw)	Starch (mg g^{-1} dw)	Nitrogen (% dw)	Carbon (% dw)	C/N ratio
30	CA	69.42	113.65	3.29	54.2	16.47
	CE	85.1 (+23)	150.31* (+32)	2.6 (-21)	67.32 (+24)	24.61* (+49)
65	CA	64.95	127.67	3.55	51.7	14.4
	CE	85.44* (+32)	175.25* (+37)	2.71* (-24)	68.0 * (+35)	24.7* (+72)
95	CA	77.51	109.03	3.46	49.42	14.93
	CE	89.34 (+15)	147.12* (+35)	2.79 (-19)	62.55 (+27)	26.05* (+74)

Values in parentheses indicate per cent change due to elevated CO_2 exposure, *difference between CA and CE significant at $p<0.05$. d.w.- dry weight of leaves.

bean grown under elevated CO_2 concentration (Prasad *et al.* 2004).

Nitrogen concentration decreased in the leaves of chickpea plants grown under elevated CO_2. The average reduction in nitrogen concentration was 21 %, and the maximum reduction (24 %) occurred at 65 DAE to elevated CO_2 (Table 2). The organic carbon concentration, which constitutes both structural and non-structural components, increased in elevated CO_2 grown chickpea plants (Table 2). The increase in organic carbon content was 24, 35, and 27 per cent at 30, 65, and 95 DAE, respectively. The increase in carbon concentration and reduction in nitrogen concentration increased the C/N ratio of chickpea leaves, which was highest (74 %) at 95 DAE. A reduction in nitrogen concentration with elevated CO_2 exposure has been reported in different crop species including legumes (Conroy 1992, Prasad *et al.* 2004, Idso and Idso 2001). The reduction in nitrogen concentration may be either due to a dilution effect as a result of greater carbohydrates accumulation or due to acceleration of plant growth under the elevated CO_2 concentration (Pal *et al.* 2004). Since this study was conducted in pots inside open-top chambers, reduction in nitrogen is likely caused by constraints to the root system, despite the dependence of chickpea on symbiotic nitrogen fixation. Higher C/N ratio due to elevated CO_2 has been reported in various investigations (Gifford *et al.* 2000, Pal *et al.* 2004).

Sucrose content, sucrose phosphate synthase activity and starch content: The sucrose content increased in elevated CO_2 grown plants and maximum increase was observed at 65 DAE (55.9 %), while, at 30 and 95 DAE it increased 33.1 and 7.2%, respectively (Fig 1). Changes in the concentration of sucrose in chickpea plants were found to be correlated with sucrose phosphate synthase (SPS) enzyme activity (Fig 2). Maximum increase in SPS activity (28%) was observed at 65 DAE but at 30 and 95 DAE it increased marginally (8.0 and 4.0%, respectively). Starch content increased by 9.7% in CO_2 exposed chickpea plants at 30 DAE compared to ambient CO_2. But the increase in starch content at 65 and 95 DAE was higher (17.39 and 16.58%, respectively) (Fig 3). Maximum SPS activity in CO_2 exposed chickpea plants was observed during the flowering stage and was associated with a high sucrose content. This indicates that under elevated CO_2, chickpea plants exhibited higher SPS activity to meet the high demand of photo-assimilates during the reproductive development. Similar changes in SPS activity are reported by Prasad *et al.* (2005) in *Phaseolus* bean exposed to CO_2 concnetration at 700 µl l^{-1} level.

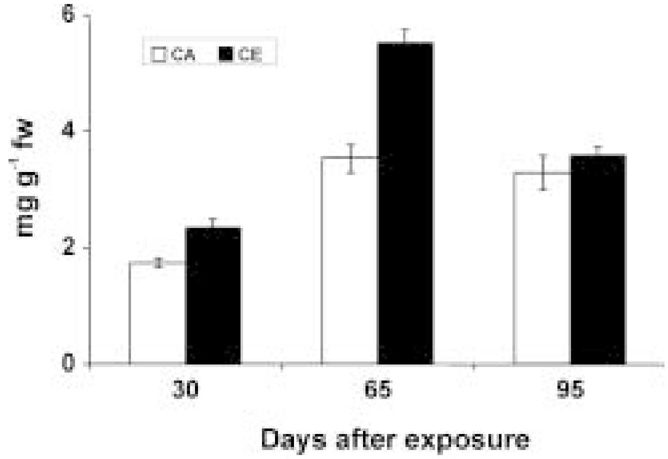

Fig. 1. Effect of elevated CO_2 on sucrose content in chickpea at different days of exposure. (CA = ambient CO_2, 370±20 µl l^{-1} and CE= elevated CO_2, 550±50 µl l^{-1}).

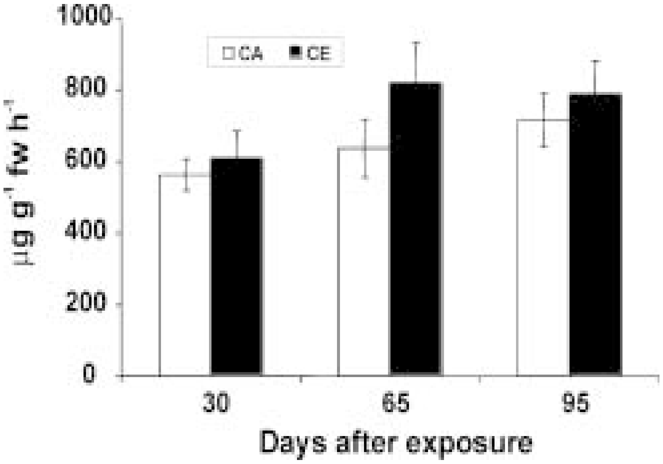

Fig. 2. Effect of elevated CO_2 on sucrose phosphate synthase activity in chickpea at different days of exposure. (CA = ambient CO_2, 370±20 µl l^{-1} and CE= elevated CO_2, 550±50 µl l^{-1}).

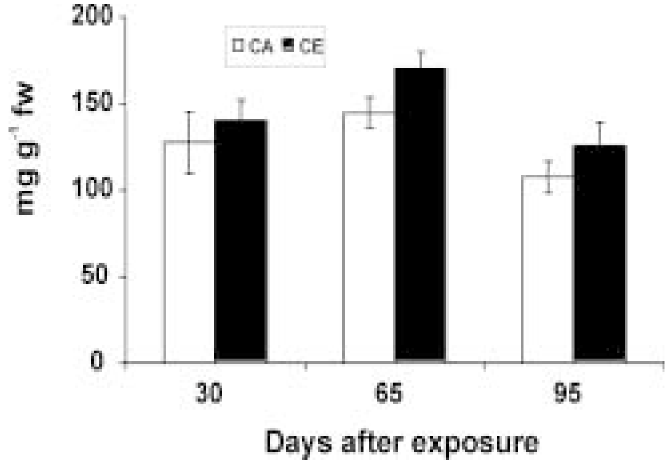

Fig. 3. Effect of elevated CO_2 on starch content in chickpea at different days of exposure. (CA = ambient CO_2, 370±20 µl l^{-1} and CE= elevated CO_2, 550±50 µl l^{-1}).

Despite the indeterminate growth habit and symbiotic nitrogen fixation, inherent in chickpeas, there was accumulation of reducing sugars and sucrose content in the leaves of elevated CO_2 grown plants. There is a possibility that restricted rooting volume in this study could be one of the reasons associated with photosynthetic acclimation. Bareet and Gifford (1995) have reported that the stimulation in the rate of photosynthesis under elevated CO_2 is reduced to half when the rooting volume is restricted compared to field experiments. Such effect of rooting volume on acclimation is probably confounded with an effect of nutrient availability on photosynthesis. The present study was conducted in pots and limited rooting volume in the pots could be one of the reasons for accumulation of carbohydrates.

Yield attributes: Increase in plant growth and dry matter accumulation in chickpea under elevated CO_2 caused an appreciable increase in plant seed yield. All the yield parameters increased due to exposure to higher CO_2 concentration and the effect was more evident on seed number per plant which increased by 28% under elevated CO_2 concentration, while the seed yield per plant increased by 15% (Table 3). The increase in number of seeds per pod was marginal (6 %) in elevated CO_2 grown chickpea plants. A similar increase in seed yield per plant under elevated CO_2 has been reported in soybean (Allen *et al.* 1991), dry bean (Prasad *et al.*

Table 3. Impact of elevated CO_2 exposure on yield attributes of chickpea plants.

Yield attributes	CA	Œ	% Change
Total pod number (plant^{-1})	36.22	41.77	+16
Total seed number (plant^{-1})	29.15	37.4*	+28
Number of seeds (pod^{-1})	0.81	0.86	+6
Total seed yield (g plant^{-1})	6.48	7.46	+15

CA = ambient CO_2, 370±20 µl l^{-1} and CE= elevated CO_2, 550±50 µl l^{-1}, *difference between CA and CE significant at $p<0.05$.

2002), peanut (Clifford *et al.* 1993, Stanciel *et al.* 2000), and cowpea (Ahmed *et al.* 1993). In this study, increased seed yield in elevated CO_2 grown chickpea plants was attributed to the significant increase in pod and seed number per plant but not due to an increase in individual seed weight. The increase in pod number per plant was due to more branches produced in plants grown under elevated CO_2 concentration.

It appears that chickpea plants respond to rising atmospheric CO_2 in terms of enhanced dry matter accumulation and seed yield. However, since nitrogen is the major protein component, reduction in its concentration may alter the grain quality of this important legume crop. Further studies are therefore, necessary to elucidate the impact of expected future global climate.

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