

चने (साइसर एराइटीनम) में जल-उपयोग एवं जड़-क्षेत्र कार्बन
गतिकी पर प्रवर्धित वातावरणीय CO₂ का प्रभाव

**Effect of Elevated Atmospheric CO₂ on
Chickpea (*Cicer arietinum* L.) Water Use and
Rhizosphere Carbon Dynamics**

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**EFFECT OF ELEVATED ATMOSPHERIC CO₂ ON CHICKPEA (*Cicer
arietinum* L.) WATER USE AND RHIZOSPHERE CARBON DYNAMICS**

By

SAURAV SAHA

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This is to certify that the thesis entitled, “**Effect of Elevated Atmospheric CO₂ on Chickpea (*Cicer arietinum*) Water Use and Rhizosphere Carbon Dynamics**”, submitted to the Faculty of Post Graduate School, Indian Agricultural Research Institute, New Delhi, by Mr. Saurav Saha, in partial fulfillment of the requirements for the award of the degree of ***Doctor of Philosophy in Agricultural Physics***, is a record of bona fide research work carried out by him under my guidance and supervision and that no part of the thesis has been submitted by him for any other degree or diploma..

I further certify that the assistance and help received during the course of this investigation has been duly acknowledged.

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*A Small Gift To
My Beloved Parents &
Shantha Mam*

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1. INTRODUCTION

Although during the past decades increases in crop productivity have matched population growth, a further understanding of how predicted environmental conditions will affect crop production in the future is a matter of major concern for worldwide food security programmes (Parry and Hawkesford, 2010, 2012; Foulkes *et al.*, 2011). This is especially relevant considering the ongoing increase in the world population, together with social changes of a diverse nature, including diets, fuels, etc. In this sense, it has been observed that food production across world needs to increase 100% by 2050 to meet the projected demands (Nakicenovic *et al.*, 2000).

The increase of atmospheric CO₂ level is thought to be due to the increased burning of fossil fuels and other anthropogenic interferences that may lead to substantial changes in the earth climate within the next century. Atmospheric CO₂ concentrations have risen from a global preindustrial level of 280 $\mu\text{mol mol}^{-1}$ to presently at 385.2 $\mu\text{mol mol}^{-1}$ (Dlugokencky *et al.*, 2010) and according to our current understanding of the global carbon cycle further increases at about 1.8 $\mu\text{mol mol}^{-1} \text{ yr}^{-1}$ (Mendelsohn and Rosenberg, 1994) to levels of between 470 and 570 $\mu\text{mol mol}^{-1}$ are expected by the year 2050 (IPCC, 2007). Currently, terrestrial vegetation is growing at a CO₂ level that is higher than in the past 26 million years. As atmospheric CO₂ is the sole source of carbon for plants, CO₂ enrichment will act as carbon fertilizer, resulting in far reaching consequences for plant production (Kimball *et al.*, 2002).

This elevated atmospheric CO₂ level is expected to affect the plant growth behavior and developmental pattern, primarily due to changes in photosynthesis and carbon assimilation pathways along with its resource use efficiency with a primer modification of its source sink relationship. Several studies have revealed that plant growth usually increases under elevated CO₂. Under elevated CO₂ concentration, potential net photosynthesis is enhanced particularly for C₃ plants (Drake *et al.*, 1997; Ainsworth and Long, 2005), resulting in increase in yield (Oliveira *et al.*, 2010) especially for short-term exposures. However, long-term CO₂ exposure experiment results have shown a reduction in potential growth due to photosynthetic acclimation under elevated CO₂ condition (Miller *et al.*, 1997; Erice *et al.*, 2006a; Aranjuelo *et al.*, 2009a). That process is often known as photosynthetic “down-regulation” (Saralabai

et al., 1997; Ainsworth *et al.*, 2003; Sanz-Sáez *et al.*, 2010) mainly attributable to a metabolic reduction due to reduced carboxylation activity (Erice *et al.*, 2006b) along with reduced amount of rubisco under elevated CO₂ condition (Urban, 2003; Aranjuelo *et al.*, 2005).

By influencing the rate of leaf expansion or canopy depth, elevated CO₂ can potentially increase LAI, thereby countering its stimulation of RUE due to enhanced growth rates (Ainsworth and Long, 2005; Saha *et al.*, 2012). Therefore, increased levels of atmospheric CO₂ may alter the structure and composition of plant communities by affecting how species respond to their physical and biological environment (Catovsky and Bazzaz, 1999). Thus, the pattern of crop water use is altered from one crop species to another. At the canopy scale, ET could be enhanced by the larger leaf area, produced in response to elevated CO₂ (Kim *et al.*, 2001). Field experiments indicate that CO₂ enrichment will also increase crop water use efficiency mainly by increasing photosynthesis and growth (Polley, 2002). Elevated CO₂ also significantly increases canopy remote sensing indices, such as, NIR/RED, PRI, chl. index, indicating a stimulation of LAI and photosynthetic carbon assimilation, as well as delayed senescence in soybean canopy (Gray *et al.*, 2010).

There is considerable uncertainty about how rates of soil carbon (C) and nitrogen (N) cycling will change as CO₂ accumulates in the Earth's atmosphere. Root biomass is known to increase under elevated CO₂ condition (Rogers *et al.*, 1994; Pritchard *et al.*, 2001; Saha *et al.*, 2011), but detailed study of root growth and functions are still lacking for nodule forming legumes (Gai-ping *et al.*, 2006). With few exceptions, rates of soil and microbial respiration are usually more rapid under elevated CO₂, indicating that greater plant growth under elevated CO₂, enhanced the amount of C entering the soil, and additional substrate was being metabolized by soil micro organisms (Donald *et al.*, 2000). For three ecosystems, it has been shown that water stability and size distribution of aggregates is affected by long-term exposure to elevated CO₂ (Rilling *et al.*, 1999). Elevated atmospheric CO₂ could potentially increase the soil aggregation due to increase in root growth (Paterson *et al.*, 1997; Rogers *et al.*, 1998), helping stabilize aggregates via enmeshment. On the other hand, the byproducts of microbial activity, such as extracellular polysaccharides of bacteria or glycoprotein glomalin of arbuscular mycorrhizal fungi, can form, with the surrounding mineral particles, an organo-mineral sheath around the cells (Chenu,

1993; Dorodnikov *et al.*, 2009) leading to an increase in macroaggregates as an indirect additional effect. Elevated CO₂ caused increase in yield and carbon uptake by all plant parts of wheat and also increased all the active carbon- pools in rhizosphere (Kant *et al.*, 2007). Besides, rhizodeposition under elevated CO₂ may stimulate mineralization of N from recalcitrant SOM pools (De Graaff *et al.*, 2011). A study of active and labile carbon-pools can help in understanding soil organic carbon dynamics on exposing plants to elevated levels of atmospheric CO₂ condition. Therefore it is important to study the changes in canopy characteristics of the plant system brought about by elevated CO₂ and its impact on plant growth, dry matter partitioning, water uptake pattern, root dynamics, active soil carbon pool and soil biophysical characteristics in important food crops

Chickpea [*Cicer arietinum* L.] a member of genus *Cicer*, tribe *Cicereae*, family fabaceae, and subfamily Papilionaceae, is the world's third most important food legume crop. Although originated in southeastern Turkey (Ladizinsky, 1975), this crop has been widely adapted to agro-climatic conditions around the world (Khanna-Chopra and Sinha, 1987). India is the principal chickpea producing country in South-East Asia with 84% share in this region (<http://www.icrisat.org/crop-chickpea.htm>, accessed 19th July 2013). Currently chickpea is cultivated in 8.56 Mha areas with total annual production of 7.35 Mt and average productivity of 858 kg ha⁻¹ (Singh *et al.*, 2010).

Chickpea has high commercial potential as food (leguminous pulse) for dry land agriculture in the semi-arid tropics where available irrigation facility is a major limiting factor, with its additional benefits of biological nitrogen fixation. Comparatively less emphasis is given to this pulse from this perspective. Therefore, it is important to study the changes in canopy characteristics of the plant system brought about by elevated CO₂ and its impact on plant growth, dry matter partitioning, water uptake pattern, root dynamics, active soil carbon pool and soil biophysical characteristics in important food crops. That will help us to know the behavior of this crop under future scenario of elevated CO₂ levels since the CO₂ will certainly have impact on its above ground and below ground processes through its altered biomass partitioning as well as altered carbon and nitrogen partition dynamics.

Only single field scale study has been conducted by Pal *et al.* (2008) to investigate the elevated CO₂ effects on chickpea but that was mostly confined to the

physiological aspects and growth characteristics of above-ground plant parts. But no study is available about the impact of elevated CO₂ on resource (water, radiation and nitrogen) use efficiencies, root and nodulation characteristics, rhizosphere C and N dynamics and quality of produce. Considering these, the present investigation was undertaken to study the effect of elevated CO₂ (~580 µmol mol⁻¹) on chickpea by using open top chambers (OTCs) with the following objectives:

1. To study the radiation and water use by chickpea crop under elevated CO₂ condition.
2. To monitor root growth behavior in chickpea under elevated CO₂ condition.
3. To study rhizosphere carbon and nitrogen pools and soil biophysical characteristics as influenced by elevated atmospheric CO₂ level.

2. BACKGROUND

Rising atmospheric CO₂ is altering global temperature and precipitation patterns, which challenges our agricultural productivity. On the other hand, rising CO₂ can provide a unique opportunity to increase the productivity of C₃ crops. Elevated CO₂ concentration influences the photosynthetic processes, while the simultaneous increase of temperature influences all aspects of crop growth and development, energy, nutrient and water availability and their use and microbial activity. Similarly, increased biomass production caused by elevated CO₂ concentration implies more carbon input into the soil which could alter soil physical properties. Therefore it is important to study the changes in microclimate of the soil and canopy of the plant system brought about by elevated CO₂ and its impact on plant growth, dry matter partitioning, root dynamics, active soil carbon pool and soil physical characteristics in important food crops.

Chickpea [*Cicer arietinum* L.] is an important winter-season pulse and a cheapest source of protein for millions of families in developing countries (Joshi *et al.*, 2002). It is the third most important food legume having a share of 14.6% in global pulse production (FAOSTAT, 2010), in the world grown on 11 million ha worldwide (15.3% of the total pulses area) adding 8.8 million tonnes to the global food basket. Asia accounts for 89.7% of global chickpea production. Within Asia, India is the largest producer of chickpea, accounting for 64% of the global chickpea production (Gaur *et al.*, 2010). Up to our knowledge domain, some field-scale research literatures are available investigating the radiation interception and RUE for chickpea, but no one under atmospheric CO₂ enrichment condition so far. Therefore, it is relevant to examine the possible changes in crop behavior for chickpea, under CO₂ enriched environment in subtropical India.

In this context, the present investigation has been planned to study the impact of elevated atmospheric CO₂ (580±20 µmol mol⁻¹) on soil and plant biophysical parameters like root water uptake, radiation use and rhizosphere carbon dynamics in chickpea (*Cicer arietinum* L.) during *rabi* (November- March) season in four open-top chambers (OTCs) at Indian Agricultural Research Institute, New Delhi, keeping

the ambient CO₂ concentration at 385 $\mu\text{mol mol}^{-1}$. The relevant literature for this present study has been given below under the following headings:

2.1 Crop growth response to elevated CO₂

Elevated CO₂ ultimately results in enhancement of photosynthesis, which increases growth rates and yields of C₃ plants (Centritto *et al.*, 2002; Drake *et al.*, 1997; Kang *et al.*, 2002; Li *et al.*, 2003) and substantial increase in leaf photosynthesis throughout the life of the crop (Garcia *et al.*, 1998). FACE experiments with soybean (Rogers *et al.*, 2004) and clover (Ainsworth *et al.*, 2003) showed stimulation in photosynthetic Carbon uptake and leaf carbohydrate content at elevated CO₂ with increased biomass. Higher atmospheric CO₂ concentration may influence positively plant production once the substrate for photosynthesis and gradient increase between the ambient air and mesophyll cells. Plants respond not only to change in surrounding CO₂ concentration, but to modifications of their microenvironment as reported in maize (Anda and Kocsis, 2008). In their crop simulation study, Song *et al.* (2013) formulated 3D canopy architecture model and kept their advent towards the possibility of the stem height, leaf width and leaf angle manipulation for *indica* rice to optimize canopy CO₂ uptake rate leading to enhanced canopy photosynthesis.

Several studies have shown that regulation of stomata by guard cells is directly affected by atmospheric CO₂ concentration (Zhu *et al.*, 1998). With few exceptions, elevated CO₂ studies show that stomatal conductance of C₃ plants is initially reduced in response to elevated CO₂, resulting in reduced transpiration and increased conservation of water. However, these responses are short-lived in some species (Bunce, 1992). Yoshimoto *et al.* (2005) reported elevated CO₂ reduced stomatal conductance and reduced transpiration combined with increased total biomass increased water use efficiency for rice in free-air CO₂ enrichment (FACE). This observation is supported by Medlyn *et al.* (2001) and Ainsworth and Long (2005). But the reduction of transpiration does not always translate to an equivalent reduction in an improved soil-water status (Field *et al.*, 1995; Wullschleger and Norby, 2001). Because elevated CO₂ often increases leaf area index (LAI) (Ainsworth and Long, 2005), which is a primary determinant of both productivity and water use (Wullschleger *et al.*, 2002a). Li *et al.* (2004a) found that the CO₂-enrichment-induced decrease in transpiration almost compensated for the increase in ET brought by the

higher leaf area under adequate N and water supply. Recent evidence from FACE experiments, where the crops are grown more naturally under the elevated CO₂, also suggests that the decrease in stomatal conductance at elevated CO₂ often translates to a proportional reduction in canopy transpiration and increased soil moisture content in soybean (Leakey *et al.*, 2009). Although at the leaf level, a given proportional increase in CO₂ concentration brings proportional increase in transpiration efficiency (by increasing photosynthetic rate and decreasing in stomatal conductance), scaling up from leaf to crop constrain the increase in net carbon gain and reduce the anti-transpiration effect of CO₂ enrichment. Peach (*Prunus persica* L.) seedlings grown under elevated CO₂ in open-top chambers showed higher assimilation rates and an increase in total dry mass. But in this experiment, stomatal conductance, total water uptake, leaf area and leaf number were unaffected by elevated CO₂ throughout the growing season (Centritto *et al.*, 2002).

Leaf area index (LAI) is defined as, the total one-sided area of leaf tissue per unit ground surface area. Leaf area index drives both the within- and below-canopy microclimate, determines canopy water interception, radiation extinction, water and carbon gas exchange and therefore is a key component of biogeochemical cycles in ecosystems (Bréda, 2003).

Differences in canopy structure, in terms of the extinction coefficient in the Beer's law and corresponding variation in LAI values for a specific crop species are the two principle factors for differences in RUE from one crop species to other. Seedlings grown under elevated CO₂, shows dramatic increases in leaf area (Kellomäki and Wang, 1997; Tissue *et al.*, 1997). McCarthy *et al.* (2007) reported the dynamics of pine canopy leaf area production and senescence were unaffected by elevated CO₂, although canopy leaf area senescence for hardwoods was slowed. On the other hand, Norby *et al.* (2003) found that there was no effect of elevated CO₂ on leaf area index for forest species. For chickpea exposed to elevated CO₂, Pal *et al.* (2008) found CO₂ exposure increased growth characteristics, viz. shoot length, total number of branches, leaf area per plant, seed yield with significant increase in leaf and shoot dry weight. Vanaja *et al.* (2007) reported that growth parameters viz., length and weight of root and shoot, root: shoot ratio, leaf area and plant weight significantly increased at 700 ppm CO₂ in black gram (*Vigna mungo* L.) using open-top Chambers. In their open top chamber study, significant increase in LAI was

reported for *Lolium perenne* (Nijs *et al.*, 1989), wheat (Tausz-Posch *et al.*, 2012), sorghum (Ottman *et al.*, 2001), *Cenchrus ciliaris* L. (Bhatt *et al.*, 2007), soybean (Dermody *et al.*, 2008), rice (De costa *et al.*, 2006; Zeng *et al.*, 2011), pigeon pea (Saha *et al.*, 2012) for different levels of atmospheric CO₂ enrichment. In contrast, reducing trend of LAI in atmospheric CO₂ enrichment was reported for maize (Kim *et al.*, 2007), sugarbeet (Manderscheid *et al.*, 2010) and *Echinochloa crusgalli* L. (Zeng *et al.*, 2011). Short term exposure of elevated CO₂ was reported to cause a significant increase in biomass production for rice without change in LAI (Imai *et al.*, 1985; Anten *et al.*, 2003). This can be further explained by means of a reduction in total leaf N in the canopy counterbalancing the increase in LAI for a given leaf N content and arguing the LAI increase under elevated CO₂ can only be happen if N uptake from the soil is simultaneously increased (Anten *et al.*, 2003). Thus, lower N availability may found to be the effective cause of suppressing the response of LAI towards elevated CO₂ (Arnone and Körner, 1995; Franklin and Ågren, 2002). Sims *et al.* (1999) also found no significant difference in total canopy leaf area with elevated CO₂ level of 700 $\mu\text{mol mol}^{-1}$ for sunflower. In their meta-analysis of studies of the free-air CO₂ enrichment (FACE), Kimball *et al.* (2002) claimed an 11% increase in peak LAI. But almost no increase in average LAI (Long *et al.*, 2004) was reviewed. For the N scarce condition, leaf N may be remobilized from older to developing new leaves in elevated CO₂, accelerating senescence of shaded foliage and thus reducing LAI (Hartz-Rubin and Delucia, 2001).

No significant effect of elevated CO₂ level was found on the LAI of the thirty-six mesocosms, each containing the community of C₃ legume, *Trifolium repens* and a C₄ grass, *Stenotaphrum secundatum*, under high P fertilization (Edwards *et al.*, 2005). Ewert (2004) reported the relatively smaller interactions between LAI and elevated CO₂ effects on canopy assimilation. Ainsworth and Long (2005) reviewed an average 21% increase in LAI for tree species but no significant change in LAI for herbaceous C₃ grasses. Weigel and Manderscheid (2012) reported about no statistically significant CO₂ effect on LAI for barley and wheat but significant reduction for ryegrass. Elevated CO₂ was found to modify the temporal extent plant LAI response by delaying LAI loss to senescence in soybean (Dermody *et al.*, 2006) and early attaining of peak LAI in pigeon pea (Saha *et al.*, 2012).

Elevated CO₂ have its own modifying effect on canopy chlorophyll concentration. Several Studies conducted elsewhere showed that elevated CO₂ exposure results in increase in fully expanded wheat leaf chlorophyll content (Demothes and Knoppik 1994), remained unaffected (Mulholland *et al.*, 1997, Manderschied and Weigel, 1997; Monje and Bugbee, 1998; Donnelly *et al.*, 2000) or decreased (Tuba *et al.*, 1994; Cheng *et al.*, 2010). The reported increase in chlorophyll a content of cotton leaves under free air CO₂ enrichment condition (Pinter *et al.*, 1994) was supported by the findings of increase in leaf chlorophylls a and b due to elevated CO₂ exposure of 600 ppm in *Cenchrus ciliaris* (Bhatt *et al.*, 2007). In contrast, leaf chlorophyll and nitrogen content of sour orange (*Citrus aurantium* L.) trees were found to reduce under elevated CO₂ exposure (Idso *et al.*, 1996). Manderscheid *et al.* (2010) reported a reduction in canopy chlorophyll content in sugar beet. Leaf chlorophyll levels were found to be decreased in response to CO₂ enrichment for tobacco (Sicher, 2011) *Betula papyrifera* and *Populus tremuloides* (Nabity *et al.*, 2012). Besides, the accelerated decline in leaf chlorophyll concentration as well as N content during senescence was found due to exposure in CO₂ enrichment for wheat (Nie *et al.*, 1995; Ommen *et al.*, 1999) and rice (Haque *et al.*, 2006). In contrast, Taylor *et al.* (2008) reported delayed senescence in populus trees with delayed decline in leaf chlorophyll content under enriched atmospheric CO₂ concentration.

The concept of canopy extinction coefficient (k), describes how the intercepted radiation is attenuated from the major beam of penetrated light within the crop canopy either by absorption or by scattering phenomenon. The canopy's radiation extinction coefficient (k) of Beer's law is defined as the average projection of leaves onto a horizontal surface. It is a function of the area and form of the leaf, the leaf inclination, the zenith angle of the sun, and the leaf's azimuth (Awal *et al.*, 2006). The light extinction coefficient (k) value quantifies the efficiency of light interception per unit leaf area index. Growth rate was negatively correlated with k for different C₃ crops (Sheehy and Peacock, 1977). Canopy constructed by more erect leaf types have smaller values of k . Radiation distribution within the crop canopy is mostly controlled by four factors, influencing radiation extinction coefficient (k) i.e. leaf optical properties (scattering coefficient for the leaves), canopy structure (leaf angle distribution), foliage amount (LAI) and incident solar angle (Sellers, 1985).

In the absence of biotic and abiotic stresses, the cumulative biomass production is linearly related with the cumulative amount of intercepted photosynthetically active radiation (PAR) by the standing crop canopy (Kiniry *et al.*, 1989; Sinclair and Muchow, 1999). The slope of this relation, known as radiation use efficiency (RUE), the most useful, efficient and diagnostic approach for quantifying crop dry matter accumulation and grain yield per unit solar radiation intercepted by the crop species. Basically, it is an indirect measure of carbon dioxide assimilation capacity of crop canopy (Muchow and Sinclair, 1994), as a function of leaf N content (Sinclair and Horie, 1989). There was a strong positive linear relationship between RUE and applied nitrogen (Shah *et al.*, 2004). Yield is a product of the total incident solar radiation over the entire crop growth period, radiation intercepted by the crop, RUE and harvest index (HI) (Monteith, 1977; Russell *et al.*, 1989) reflecting the efficiency of the conversion of the intercepted light into dry matter. Leaf arrangement and inclination are the major determining factor for modeling of differential responses between plant growth and the physical environment. Both, the extinction coefficient and the RUE are considered to be the most appropriate and effective parameters in use to incorporate in crop simulation models (Jones and Kiniry, 1986; Ritchie *et al.*, 1988; Amir and Sinclair, 1991; Brisson *et al.*, 2003).

In the absence of growth limitations from water stress, nutrient deficiency, pests or diseases, RUE is believed to be constant from crop emergence until senescence due to sharp decrease in LAI as the crop matures (Monteith and Elston, 1983). The value of RUE did not vary significantly for a specific crop species with variation in environmental conditions for cereals (Gallagher and Biscoe, 1978), rice (Horie and Sakuratani, 1985), maize (Lindquist *et al.*, 2005) and for soybean, mungbean (*Vigna radiata*) and cowpea (*Vigna unguiculata*) (Muchow *et al.*, 1993), due to the constancy of the canopy photosynthetic efficiency and the strong relation between photosynthesis and respiration process by the wheat crop (Hay, 1999). Kumar *et al.* (1996) analyzed the need to incorporate the effect of weather parameters on RUE in the algorithms for calculation of biomass in crop simulation models. In contrast, to the findings of Campbell *et al.* (2001) about need for change in RUE value for differing LAI during pre- and post-anthesis periods of rice, O'Connell *et al.* (2004) reported that seasonal weather conditions had no effect on the magnitude of RUE for irrigated wheat, pea and mustard. Declining trend of RUE was reported by

Stockle and Kiniry (1990) and Collino *et al.* (2001) in crop plants exposed to a high vapor pressure deficit. Water stress was found to be a major factor for decrease in RUE, especially during grain filling in pigeon pea (Hughes and Keatinge, 1983), pearl millet (Ong and Monteith, 1985), fababean (Ridao *et al.*, 1996), peanut (Collino *et al.*, 2001). But phosphorus deficiency was found to have no effect on maize RUE (Plènet *et al.*, 2000), even with a significant reduction in above-ground biomass accumulation. Increased diffuse radiation coupled with decreased total irradiance may increase crop RUE value (Sinclair *et al.*, 1992; Choudhury, 2000) along with non-uniform specific leaf N distribution (Sinclair and Shiraiwa, 1993). Radiation-use efficiency may also be affected by mean daily temperature for sorghum (Hammer and Vanderlip, 1989), maize (Andrade *et al.*, 1993) and wheat (Manderscheid *et al.*, 2003). Temperature appeared to be an important factor determining RUE under predominantly cloudy conditions (Choudhury, 2000). The value of RUE even vary with crop phenology in maize (Giauffret *et al.*, 1991), following a consistent sigmoidal pattern in field pea (Lecoeur and Ney, 2003).

The impact of elevated atmospheric CO₂ on radiation use efficiency of different crop species is essentially needed to evaluate the crop responses to changing climate using RUE based crop simulation models. Several reports are available examining the elevated CO₂ affects on canopy radiation interception and its use efficiency. RUE was found to be 40% higher in 800 $\mu\text{mol mol}^{-1}$ than in 330 $\mu\text{mol mol}^{-1}$ atmospheric CO₂ level for soybean canopy (Acock *et al.*, 1985). Elevated CO₂ (700 $\mu\text{mol mol}^{-1}$) was found to increase the RUE by 30% in well-watered peanut stands (Clifford *et al.*, 1993) offsetting the negative effects of water deficit on peanut RUE. Dependence of RUE on temperature, as well as atmospheric CO₂ concentration was modeled by Haxeltine and Prentice (1996). Higher CO₂ assimilation was found to be positively correlated with RUE in peanut (Bennett *et al.*, 1993), that enabled the crop plants to accumulate more dry matter more efficiently in expense of intercepted canopy radiation, resulting in a net increase in RUE for several cultivated crop species (Mulholland *et al.*, 1998; Pinter *et al.*, 1996; Rudorff *et al.*, 1996; Drake *et al.*, 1997; Monje and Bugbee, 1998; Lin *et al.*, 1998; Hui *et al.*, 2001; Manderscheid *et al.*, 2003; Dermody *et al.*, 2008; Manderscheid *et al.*, 2009). Manderscheid *et al.* (1997a) showed that the elevated CO₂ effect on dry matter production of clover does not depend on temperature but on the intercepted radiation only. A curvilinear increase in

RUE between 380 and 660 $\mu\text{mol mol}^{-1} \text{CO}_2$ in white clover from 2.7 g MJ^{-1} to 3.9 g MJ^{-1} was attributed to the positive CO_2 effect on the quantum use efficiency of photosynthesis. Sakai *et al.* (2001) reported that the increase in rice biomass by elevated CO_2 results in more increase in carbon gain at early rather than later growth stages in rice. Higher LAI values, along with low light extinction coefficients (k) contributed to higher RUE in switchgrass (Kiniry *et al.*, 1999). For closed forest canopy, increasing atmospheric CO_2 was found to stimulate biomass accumulation significantly with increase in RUE value (Delucia *et al.*, 2002). The increase in LAI for tree species may result in more rapid canopy closure, which would affect light interception (Ward and Strain, 1999). Increase in LAI under elevated CO_2 was found to increase canopy light absorption for field-grown wheat (Pinter *et al.*, 1996; Mulholland *et al.*, 1998; Batts *et al.*, 1998) and cotton (Pinter *et al.*, 1994). In contrast, some reports are available informing about no effect on canopy light absorption of elevated CO_2 condition either with increase in LAI in wheat (Smart *et al.*, 1994; Rudorff *et al.*, 1996), rice (De Costa *et al.*, 2006) and soybean (Dermody *et al.*, 2008) or with reduced leaf area (Monje and Bugbee, 1998). Smart *et al.* (1994) reported a 30% increase in LAI under elevated CO_2 with increased radiation penetration in wheat canopies. They indicated a 17% reduction in extinction coefficient (k) under elevated CO_2 . In contrast, elevated CO_2 was reported to modify the canopy structure by shifting the distribution of leaf area within the canopy so that more leaf area was concentrated near the top of the canopy, resulted in as much as a 50% reduction in photon flux density in the upper portions of the elevated CO_2 exposed sunflower canopy (Sims *et al.*, 1999). Thus, CO_2 induced increase in LAI may be nullified by subsequent reduction in the extinction coefficient. A study by Manderscheid *et al.* (2003) on the effect of CO_2 enrichment on growth and daily radiation use efficiency of wheat in relation to temperature and growth stage confirmed the theoretically expected decrease of RUE with temperature, when crop growth depends on source activity. They questioned the use of a constant multiplier of RUE to model effects of increased atmospheric CO_2 concentrations on crop growth. They suggested the use of a temperature dependence of RUE which is modified by atmospheric CO_2 concentrations. Norby *et al.* (2003) found no effect of elevated CO_2 on absorbed PAR with increase in net primary productivity with increase in light use efficiency under elevated CO_2 . Manderscheid *et al.* (2009) reported the ameliorative effect of CO_2

enrichment on decreased seasonal radiation absorption by the green canopy under limited nitrogen supply. De costa *et al.* (2006) found a sharp decrease in rice RUE, growing under elevated CO₂ during the post-heading period, may be explained as an effect of reduction in leaf nitrogen concentration. Weerakoon *et al.* (2000) reported about the indirect effect of elevated CO₂ increasing light interception through increasing total nitrogen uptake resulting in higher LAI mostly at early crop growth stages, along with 35% increase in RUE for *Oryza sativa* L. cv. IR72) in open top chamber. Diurnal variation of RUE was found to be low at noon and high in the morning or afternoon for Sunflower (*Helianthus annuus* var. Mammoth) canopy along with higher value of RUE under elevated CO₂ (Hui *et al.*, 2001). In their open-field FACE experiment, CO₂ enrichment was found to increase in energy yield of oilseed rape (Högy *et al.*, 2010b). And for open top chamber grown pigeon pea (*Cajanus cajan* L.), change in canopy extinction coefficient was found to be dominated over increased LAI, ultimately resulted in less radiation interception by erect crop canopy, contributing to 52.3%; Increase in RUE under elevated CO₂ in open top chamber condition (Saha *et al.*, 2012).

So there are three contrasting possibilities contributing to increase in RUE under elevated CO₂ condition. As it is the well-established fact, that total crop biomass increases significantly under elevated CO₂ condition. But increased LAI may increase the canopy radiation interception, thus lowering the magnitude of increase in RUE value under elevated CO₂. Otherwise, modification in leaf orientation and canopy architecture may compensate the effect of increased LAI resulted no change or even decrease in canopy radiation interception under atmospheric CO₂ enrichment, thus modifying the crop RUE value. Our work was aimed to evaluate the best possibilities among these three. One of the traits contributing to differences in species for plant growth rate or RUE is the leaf orientation. It would be interesting to find out how the radiation interception and its utilization changes due to elevated CO₂ by affecting the canopy structure. Also, calculation of light extinction coefficient value (k) in Beer-Lambert formula to quantify efficiency of light interception per unit leaf area index and RUE values of crop canopy exposed to elevated CO₂ will be useful in simulation models aimed at predicting crop productivity for future scenarios of climate change.

2.2 Crop water use and its efficiency

It is envisaged that the evapotranspiration and water use efficiency of crop will be altered under climate change (Thomas, 2008) as it is influenced by elevated CO₂. Growth-chamber experiment shows that water use efficiency in terms of shoot biomass and grain yield were increased under elevated CO₂, because the magnitude of the increase in shoot dry weight and grain yield was greater than that of the total consumption of water under elevated CO₂ conditions (Wu *et al.*, 2004). In their experiment, under high soil moisture, wheat crop exposed to elevated CO₂ consumed more water during the entire growth season except at dough stage. A small reduction in ET in wheat grown under well-watered and high soil N conditions was observed under elevated CO₂ (Hunsaker *et al.*, 2000). The small decrease in water consumption (ET) combined with relatively higher yields under increased CO₂ is expected to result in an increase in water use efficiency of the crop. There is overwhelming evidence that growth at elevated CO₂ reduces stomatal conductance and that this instantaneous response is maintained with time. The fraction of available water that is used by crops is likely to increase with CO₂ concentration because of greater root growth and faster canopy closure (Polley, 2002). However, Kammann *et al.* (2005) found no significant trend towards higher soil moisture since the start of the CO₂ enrichment experiment. Nevertheless, a small trend towards water savings was observed in the final year. This became more prominent when the top soil dried out. The soil under elevated CO₂ became wetter compared to the ambient soil. Increase in soil moisture under elevated CO₂ could temporarily relieve plants from water stress (Widodo *et al.*, 2003; Zavaleta *et al.*, 2003; Li *et al.*, 2004a). However, warming-induced reductions in soil water availability may attenuate effects of elevated CO₂ (Niklaus and Körner, 2004). Increases in plant growth under elevated CO₂ might cause a rapid depletion of other limiting resources (Koch and Mooney, 1996; Pospisilova and Catsky, 1999) and warming could increase evapotranspiration, thereby depleting soil water. The response of plant communities to changes in resource availability is often mediated by species composition, particularly given the range of growth strategies, demography and productivity possessed by diverse plant communities (Niklaus *et al.*, 2001; Reich *et al.*, 2004). When seasonal temperatures were greatest, soil moisture contents were lowest and warming had the greatest impact on soil moisture (Dermody *et al.*, 2008). In a FACE experiment, cotton ET measured using three independent approaches: soil

water balance (Hunsaker *et al.*, 1994), sap flow gauges (Dugas *et al.*, 1994) and energy balance (Kimball *et al.*, 1994), did not significantly change with increased CO₂ to 550 $\mu\text{mol mol}^{-1}$ level. Seasonal ET (estimated using soil water balance) decreased by 4.5 and 5.8% in a well-watered treatment; but increased by an average of 4.8 and 0.9% under water-stress for the 2 years, respectively (Hunsaker *et al.*, 1996). It is found eggplants grown in a high CO₂ environment might be efficient in water use under soil water shortage conditions (Sarker and Hara, 2009). Schapendonk *et al.* (1997) reported that, though the average water use efficiency was increased at elevated (doubled) CO₂, but the higher water use efficiency was fully compensated for by a higher leaf area index, as they found total transpiration on a canopy basis was equal for the both ambient and elevated CO₂ concentrations. Overall, the effects of warming and soil moisture were more influential (i.e. both positively and negatively) on species and community-level responses than were the effects of elevated CO₂ (Engel *et al.*, 2009).

Cruz *et al.* (1997) found that under elevated CO₂, nitrate grown plants had a larger content of sucrose in both roots and shoots while no significant difference was observed in the content of sucrose in ammonium grown plants, whether under ambient or enriched carbon dioxide. It was concluded that the differences in soluble carbohydrate contents could, at least partly, account for differences in root and shoot morphology. Fernandez *et al.* (2002) reported that soluble sugar and starch contents decreased in cassava plants with time under elevated CO₂ and the decrease in starch content coincide with the beginning of root mass increase.

Earth's terrestrial ecosystem, containing 1500–1600 Pg C in organic matter, is about 3 and 4 times as large as the C content in the atmosphere and terrestrial biosphere, respectively (Lal *et al.*, 1997; Stevenson and Cole, 1999; Lal, 2004). Given the size of the soil C pool, a very slight increase in soil C content could cause meaningful sequestration of excess atmospheric CO₂. Besides, human activity has also altered the global biogeochemical cycling of carbon (C) and nitrogen (N) by increasing atmospheric CO₂, accelerating rates of atmospheric N deposition, and decreasing plant diversity (Chapin *et al.*, 2000).

2.3 Soil carbon fractions and related enzyme activity

Soil is a living dynamic, non-renewable, resource and its conditions influence food production, environmental efficiency and global balance (Dick, 1997; Doran and Zeiss, 2000). Soil is considered to be a potential sink of atmospheric CO₂ and the most stable largest terrestrial carbon pool, which store approximately 80% of global terrestrial C (Nielsen *et al.*, 2011). Capacity of soils to store and sequester carbon depends on the balance between rates of carbon input from plants relative to CO₂ released from microbial decomposition. Plant input may be characterized by accumulation of soil organic matter due to humification after plant death and rhizodeposition process (Kuzyakov and Domanski, 2000). Changes in community composition, and the loss of species with an ability to facilitate specialized soil processes related to C cycling, as a result of global changes, may have larger impacts on soil carbon dynamics. There is considerable uncertainty about how rates of soil carbon and nitrogen cycling will change as CO₂ accumulates in the earth's atmosphere. Soil has been referred to as a major biosphere reservoir of C, with carbon content of two times that of the atmosphere and three times that of vegetation globally (Lohila *et al.*, 2003).

Soil organic matter is the most important reservoir of terrestrial organic C and minor changes in the balance may have a significant impact on the climate. Increased carbon storage in ecosystems due to elevated CO₂ may help to stabilize atmospheric CO₂ concentrations and slow global warming. Many field studies have found that elevated CO₂ leads to higher carbon assimilation by plants, and others suggested that this can lead to higher carbon storage in soils. The mineralization activity of soil microorganisms is the key regulator for carbon release as CO₂ from soil to atmosphere. The effects of elevated CO₂ on rates of net N mineralization were less distinct, and the apparent availability of N was not affected (Ross *et al.*, 1995). Short term stimulation of initial carbon mineralization (Bazot *et al.*, 2006; Hagedorn and Machwitz, 2007), along with increase in potentially mineralizable carbon in total soil carbon was reported under elevated CO₂ (Williams *et al.*, 2004). Gorissen (1996) found decomposition rates decreased by more than 30% in 64-day laboratory incubations of roots which had been grown at high CO₂ and low nitrogen availability due to increase in root C:N ratio (Hungate *et al.*, 1997a). Norby *et al.* (2001)

concluded that any change in decomposition rates resulting from elevated CO₂ exposure of plant are small when compared to other potential impacts of elevated CO₂ on carbon and N cycling. Depending on the type and quality of litter, the decomposability of residues from plants grown under elevated CO₂ concentration can be either reduced (Gorissen, 1996, Viswanath *et al.*, 2010; De Graaff *et al.*, 2011), enhanced (Sowerby *et al.*, 2000; Bazot *et al.*, 2006; Taneva and Gonzales-Meler, 2008; Peralta and Wander, 2008) or remain unaffected (Gorissen and Cotrufo, 2000; Hoorens *et al.*, 2003; De Graaff *et al.*, 2006) as compared to litter produced under ambient CO₂. Decomposition of plant materials is also controlled by N availability in the soil under elevated atmospheric CO₂ condition (Pendall *et al.*, 2004a; Viswanath *et al.*, 2010).

Elevated CO₂ is expected to have changes in rates of formation or decay of soil organic carbon with its substantial feedbacks on the atmosphere's concentration of CO₂. Meta-analyses indicate that elevated atmospheric CO₂ can produce small but measurable increases in soil carbon storage, but contrary results also exist (De Graaff *et al.*, 2006). After a decade of exposure, elevated atmospheric CO₂ had no effect on soil carbon stocks in *Lolium perenne* and *Trifolium repens* grasslands at the Swiss FACE experiment (Van Kessel *et al.*, 2006). 8-year open-top chamber experiment on native tall grass prairie in Kansas (USA) indicated increasing soil carbon stocks under elevated CO₂ (Jastrow *et al.*, 2005) resulting in a negative feedback on the increasing CO₂ concentration in the atmosphere (van Ginkel *et al.*, 1997). Supporting the findings of Hungate *et al.* (1996), recent literature reviews suggest that elevated atmospheric CO₂ is sometimes followed by an increase in soil carbon stocks (Jastrow *et al.*, 2005; Luo *et al.*, 2006) or remain unaffected in nutrient-poor native terrestrial ecosystems (Arnone and Körner, 1995). Understanding the below-ground processes will help in determining the contribution of soil carbon storage potential to atmospheric CO₂ concentrations (Norby *et al.*, 1994). To explain the no stimulation of allocation of recently assimilated carbon to the rhizosphere and no increase in sequestration of carbon in the soil of their nine year FACE enrichment, Bazot *et al.* (2006) concluded that the pattern of carbon allocation in the plant-soil ecosystem was mostly governed by plant physiological status. Hungate *et al.* (1997b) suggested a greater increase in carbon cycling than in carbon storage in grasslands under elevated

CO₂. Elevated CO₂ caused increase in yield and carbon uptake by all plant parts of wheat and also increased all the active carbon- pools in rhizosphere (Jastrow *et al.*, 2005; Kant *et al.*, 2007) and produced changes not only in below-ground carbon allocation that drives soil carbon accumulation but also in root turnover (Fitter *et al.*, 1996). Xie *et al.* (2005) reported increase in C:N ratio, and carbon input to the soil under elevated CO₂ and hence provides opportunities to sequester atmospheric CO₂–C in soils to mitigate anthropogenic CO₂. In contrast, six years of experimental CO₂ doubling showed a reduction in soil carbon in a scrub-oak ecosystem offsetting 52% of the additional carbon, accumulated at elevated CO₂ in aboveground due to higher plant growth and coarse root biomass. The reduction of this fixed carbon was driven by altering in soil microbial composition and activity converting soil system from a potential carbon sink to become a carbon source (Carney *et al.*, 2007). However, this increased carbon input to the soil can be counteracted by enhanced decomposition of old soil organic matter (priming effect), thus limiting the carbon sequestration potential under increasing atmospheric CO₂ concentrations (Niklaus and Falloon, 2006). The increased below-ground carbon allocation and greater root production can be direct contributors to soil carbon accrual under CO₂-enrichment (Luo *et al.*, 2006; De Graaff *et al.*, 2006) along with reduced decomposition of plant-derived carbon (Marhan *et al.*, 2008) leading to enhanced carbon sequestration under elevated atmospheric CO₂ concentration. Li *et al.*, (2004b) suggested that under elevated atmospheric CO₂, carbon sequestration in paddy soils is possible, only when the retardant effect of the labile plant-derived carbon on decomposition of the old soil carbon prevails for a long duration.

Zak *et al.* (1993) reported that labile carbon and microbial biomass carbon in the rhizosphere of plants grown under elevated CO₂ was greater than ambient condition. It has been reported that water stability and size distribution of aggregates are also affected by long-term exposure to elevated CO₂ (Rilling *et al.*, 1999). Organic carbon in the surface forest soil increased linearly during five years of exposure to elevated CO₂ and the CO₂-stimulated increases in soil organic carbon was found to be allocated to rapidly cycling, labile pools with little long-term stabilization (Lichter *et al.*, 2008). In contrast, from their $\delta^{13}\text{C}$ isotopic study, Pendall and King (2007) reported a potential for carbon accumulation in near-surface soil carbon pools under elevated CO₂. Anderson *et al.* (2011), in their six year FACE experiment, showed a

slightly but significantly increase (around 10.0%) in soil TOC and total N content under elevated CO₂. No differences in soil C and N content were found after 3 years of CO₂ enrichment (Rice *et al.*, 1994). Ecosystem C:N ratio was reported to be widened, although total carbon and nitrogen content was found to be increased with slight increase in mineral soil C:N ratio with no effect of elevated CO₂ on the O horizon C:N ratio (Finzi *et al.*, 2001). Both carbohydrate carbon and labile carbon increased due to exposure of elevated CO₂ in wheat (Kant *et al.*, 2007). Exposure to elevated CO₂ was failed to increase particulate organic matter carbon (Peralta and Wander, 2008). In their meta-analysis study, Sillen and Dieleman (2012a) concluded a lower potential for increase in soil carbon content with increase in root biomass in managed grassland (Sillen and Dieleman, 2012b) under elevated CO₂. Chen *et al.* (2012) documented no significant effect of elevated CO₂ on total organic carbon, particulate organic carbon, readily oxidizable organic carbon after 4 years of CO₂ enrichment with increase in microbial biomass carbon and soil respiration, but with N addition it resulted in an increase in soil particulate organic carbon and readily oxidizable organic carbon with no significant effect on soil microbial biomass carbon.

With few exceptions, soil and microbial respiration rates were higher under elevated CO₂ (Moscatelli *et al.*, 2005; Li *et al.*, 2010) indicating that greater plant growth under elevated CO₂ enhanced the amount of carbon entering in to the soil, and additional substrate was being metabolized by soil micro-organisms (Donald *et al.*, 2000). Increased atmospheric CO₂ had significant effects on the microbial community composition of soil microbes (Janus *et al.*, 2005). Bacterial diversity was found to be unaffected but fungal biomass was stimulated by the elevated CO₂ treatment (Lipson *et al.*, 2005), due to slowly changing substrate quality (Kandeler *et al.*, 2006). Nitrate reducing bacteria were reported to decrease significantly with elevated CO₂ (Lesaulnier *et al.*, 2008). In contrast, Van Groenigen *et al.* (2006) found that the contribution of saprophytic fungi and bacteria to SOM was largely unaffected by elevated CO₂. Quantitative and qualitative changes of plant C input into the soil at elevated CO₂ were reported to affect the soil microbial community functioning, but not their total content (Dorodnikov *et al.*, 2009). Sowerby *et al.* (2000) suggested that increased soil microbial biomass rather than microbial activity was responsible for significant increase in *in-situ* soil respiration under elevated CO₂ with its greater potential to degrade plant materials. Contradictory findings of no change in microbial

biomass carbon under elevated CO₂ (Kandeler *et al.*, 2006; Carney *et al.*, 2007; Lesaulnier *et al.*, 2008; Kassem *et al.*, 2008; Anderson *et al.*, 2011; Manna *et al.*, 2013) was also reported. Drigo *et al.* (2008) concluded that the main effects of elevated atmospheric CO₂ on soil microbiota occur via plant metabolism and root secretion, especially in C₃ plants, thereby directly affecting the mycorrhizal, bacterial, and fungal communities in the close vicinity of the root, but little or no direct effect on the microbial community of the bulk soil. Some studies have documented increase in below-ground plant productivity and metabolism with increased atmospheric CO₂, suggesting that a significant portion of extra carbon is transferred to root systems, and ultimately to the soil microbial community and carbon pools (Pendall *et al.*, 2004b; van Groenigen *et al.*, 2006; Hill *et al.*, 2007). Elevated CO₂ increased respiration (due to increasing root respiration) and enhanced priming of soil organic matter (SOM) and may result in ecosystem C losses (Carney *et al.*, 2007). Under to elevated CO₂ concentrations, nitrogen deposition could alleviate increasing nitrogen limitation experienced by plants exposed to increased rate of C influx to the belowground soil compartment as a consequence of elevated CO₂ concentration. But nutrient amendment was found to be least influencing factor to greatly alter microbial substrate use in soil and also promoted decomposition processes in elevated CO₂, reducing the potential for increased soil C storage (Sillen and Dieleman, 2012a).

Soil enzymes are the most effective sensors of existing soil physico-chemical health. Change in population dynamics of soil biota and their activity is the most effective modulator of soil enzyme activity. Still today very less information is available on soil enzyme responses to atmospheric CO₂ concentration enrichment. Changes in microbial activity had been shown by an increased dehydrogenase activity in *Bromus madritensis* monocultures growing at elevated CO₂, with an associated increased SOM decomposition rate (Dhillon and Roy, 1996). Manna *et al.* (2013) reported about insensitivity of elevated CO₂ to dehydrogenase, fluorescein diacetate (FDA) activity in rice. Li *et al.* (2010) reported about a 19% increase in dehydrogenase activity under elevated CO₂ affect in Mongolian oak ecosystem. Incubated rice soils at elevated CO₂ concentrations showed a significant increase in dehydrogenase activity with its maxima at 600 µmol mol⁻¹ level of elevated CO₂ (Das *et al.*, 2011). Saha *et al.* (2011) reported a significant increase in soil dehydrogenase and FDA hydrolysis activity in pigeon pea, grown under enriched CO₂ environment.

Ebersberger *et al.* (2003) and Kandeler *et al.* (2006) reported a sharp significant increase in alkaline phosphatase activity in grassland soils during spring due to CO₂ enrichment effect resulting from higher soil moisture and increased root biomass with subsequent stimulation of N-mineralization. Ruifang *et al.* (2007) and Manna *et al.* (2013) reported no change in acid phosphates activity for enriched CO₂ treatment in subalpine forest and rice, respectively. In contrast to this finding, Xuexia *et al.* (2006) and Guenet *et al.* (2012) reported about elevated CO₂ induced increase in the acid phosphatase activity in grassland soil. In P deficient solution culture, elevated CO₂ increased phosphatase activity released from root cells rather than root associated microorganisms and suggested increase in phosphatase activity was a elevated CO₂ mediated response (Moscatelli *et al.*, 2005). Evaluating the effect of five years CO₂ enrichment, elevated CO₂ did not significantly change the activities of β -glucosidase and phosphatase activity in bulk soil but decreased their activities in microaggregate. But after substrate addition both of the enzymes activity was found to be 1.7 – 1.8 times higher under elevated CO₂, resulting from accelerated carbon turnover in soil under elevated CO₂ (Dorodnikov *et al.*, 2009). Moscatelli *et al.* (2005) and Finzi *et al.* (2006) reported about the increasing trend of soil β -glucosidase activity in free air CO₂ enrichment. Xuexia *et al.* (2006) confirmed the increase in β -glucosidase activity only under high N fertilization in a rice-wheat rotation.

2.4 Soil CO₂ flux

Carbon dioxide is released from the soil through soil respiration, which includes three major biological processes, viz., microbial respiration, root respiration and faunal respiration primarily at the surface soil and chemical oxidation which could be pronounced at higher temperatures. Root carbon reserves are mainly used for respiration by remaining organs (root and nodules), while nitrogen reserves are used to support shoot regrowth (Avise *et al.*, 2003). Soil temperature and root surface area are the major controlling factors for soil CO₂ efflux (Gough and Seiler, 2004). The increasing rate of surface soil CO₂ efflux under elevated CO₂, were mainly attributed to fine-root turnover and exudation (Hungate *et al.*, 1997b; Norby *et al.*, 2001). Elevated CO₂ stimulates gross rates of ecosystem carbon fluxes, but does not necessarily enhance net carbon uptake, and that carbon cycle responses in semi-arid grasslands are likely to be more sensitive to changes in precipitation than atmospheric

CO₂ (Bachman *et al.*, 2010). However, elevated CO₂ has been shown to increase (Pendall *et al.*, 2001; Peralta and Wander, 2008; Deng *et al.* 2010) or to suppress soil respiration in some cases (Ineson *et al.*, 1998) or to have no effect in others (Johnson *et al.*, 2001). Soil CO₂ efflux can be highly variable on temporal and spatial scales within a single field experiment (Xu and Qi, 2001) and therefore, even relatively large increase in soil efflux under elevated CO₂ may not be statistically significant (Zak *et al.*, 2000). Some of the variation among individual studies may be due to differences in plant species, experimental conditions, or methods used for the determination of CO₂ efflux (Wan *et al.*, 2007). Taneva *et al.* (2008) suggested higher soil respiration rates along with accelerated decay rates and limited soil carbon accumulation under elevated CO₂.

2.5 Root growth study

Roots have been found to contribute more carbon to the soil organic carbon pool than surface residues (Johnson *et al.*, 2007) as the soil carbon response to elevated CO₂ was attributed to the root response, since there was a lower potential for increase in soil C content when root biomass was more responsive to CO₂ elevation (Sillen and Dieleman, 2012b). Elevated CO₂ had its substantial effects on root system architecture, micromorphology and physiology, resulted in an increase in root length, root dry weight, root diameter, stele diameter, cortex width, shoot and root weight ratios with no increase in root numbers (Rogers *et al.*, 1992). Specific root length (length g⁻¹ root) was also influenced by soil moisture and above-ground crop growth (Anderson *et al.*, 2007). Variability in shoot and root traits is associated with variation in the functional properties of those structures, such as tissue metabolic demand and acquisition efficiency of light and CO₂ by leaves and water and nutrients by roots (Derner and Briske, 1999; Farley and Fitter, 1999). With few exceptions (Erice *et al.*, 2006b), root biomass is normally increases under elevated CO₂, but detailed study of growth and functions of this parameter are lacking (Rogers *et al.*, 1994). It is reported that root biomass would increase with increased atmospheric CO₂, as observed in grasslands (Hungate *et al.*, 1997b; Niklaus *et al.*, 2001; LeCain *et al.*, 2006). However it is argued that effect of elevated CO₂ is to make root heavier, but translation of the increased mass into root system with increase in length has not been evidenced (Reddy *et al.*, 1994). Length and weight of roots, root:shoot ratios were significantly

increased at elevated CO₂ in black gram (Vanaja *et al.*, 2007) and root weight, volume, and the root: shoot ratio (Crush, 1993). Long-term FACE experiment did not show any significant effect of elevated CO₂ on total root biomass, fine root biomass and root mass fraction in graminaceous species, but showed a significant decrease of 28% in root mass fraction in forbs. An increase in fine root production coupled with increase in fine root turnover rate impacts the C:N dynamics and the soil organic carbon build up in annuals and herbaceous species (Norby *et al.*, 2001). Canadell *et al.* (1996) reported about the complete down regulation of net CO₂ uptake after several years of elevated CO₂ exposure with a greater proportion of fixed carbon is generally allocated to below-ground, potentially increasing the capacity of below-ground sinks and some of the increased capacity of these sinks may lead to increased long-term soil carbon sequestration. The roots would be relatively larger and will have more uptake ability (Pritchard *et al.*, 1999) in a high CO₂ concentration environment. Elevated CO₂ was reported to significantly increases later root number, total root length, root surface area, root diameter and root volume (Rogers *et al.*, 1994; de Graaff *et al.*, 2006; Saha *et al.*, 2011). As much as 52% increase in root growth is observed in some tree species (Tingey *et al.*, 2000). Elevated CO₂ enhanced the activity of nitric oxide synthase that promoted production of NO, which was involved in lateral root formation under elevated CO₂ (Wang *et al.*, 2013). Root turnover rates in tree species also varied from a decrease (Iversen *et al.*, 2008) to an increase (Tingey *et al.*, 2000; Norby and Zak, 2011). The response may vary from one species to other. The species specificity of belowground response under elevated CO₂ vary with soil depth but that could underestimate the degree and magnitude of change under monoculture than that of polyculture in terms of root specific surface area, root biomass and annual turnover (Smith *et al.*, 2013).

Rhizosphere processes play an important role in carbon sequestration and nutrient cycling in terrestrial ecosystems (Van veen *et al.*, 1991) and the rhizosphere has been identified as one of the key fine-scale components of the global carbon cycle (Coleman *et al.*, 1993). The direct effects of increasing CO₂ on plants includes the changes in leaf chemistry of plants due to increased starch accumulation (Kuehny *et al.*, 1991) through increased carbon fixation (Leakey *et al.*, 2009; Manderscheid *et al.*, 2009) and increasing concentration of phenolic compounds such as lignin and tannins (Ebersberger *et al.*, 2003). This was resulting in greater carbon allocation to both

below (Rogers *et al.*, 1994; Cotrufo and Gorissen, 1997) and above ground plant parts along with an increase in the leaf C:N ratio (Pal *et al.*, 2003) with reduced leaf stomatal conductance with higher soil water content (Rabha and Uprety, 1998; Ainsworth and Long, 2005). This controlling of the soil microbial activity through osmotic regulations alters the supply of nutrients. Higher atmospheric CO₂ increases the allocation of photosynthates to roots with more vigorous root growth and increased rhizodeposition (Pendall *et al.*, 2004b; van Groenigen *et al.*, 2006; Anderson *et al.*, 2011), as long as other soil nutrients were not limited (Johnson *et al.*, 2007) thus resulting in the enrichment of soil carbon pools (Lagomarsino *et al.*, 2008). According to Uselman *et al.* (2000), the CO₂ fertilization effect' on N-fixing trees suggests that N exudation from roots could play a significant but minor role in sustaining increase in forest growth, and thus C storage, in a CO₂ enriched atmosphere. It is because root exudation of organic carbon as well as N appears to have little potential to contribute to long-term soil C sequestration. CO₂-induced stimulation in rhizodeposition of sugars and malate was also reported with release of nod-gene-inducing flavonoids (Haase *et al.*, 2007). Pregitzer *et al.* (1995) found fine root growth rates increase and lifespan decrease due to elevated CO₂, with little change in litter quality. Janssen (1996) established a relationship, suggesting the fraction of mineralized organic N is linearly related to substrate C:N ratio for equally decomposable materials. The C:N ratio of the plant litter reaching the soil can increase (Norby *et al.*, 2001; Pendall *et al.*, 2004b) leading to nitrogen (N) limitations for microbes with consequences of altered decomposition rates of SOM (Peralta and Wander, 2008). Increased nutrient availability in the rhizosphere by stimulation of N release from recalcitrant soil organic matter pools through enhanced rhizodeposition under elevated CO₂ was reported by De Graaff *et al.* (2006). Soil microorganisms appeared to shift from consuming older soil organic carbon to utilizing easily degraded rhizodeposits derived from increased root biomass under elevated CO₂. In contrast reduction in soil nitrogen mineralization (Hungate, 1999) with increased retention of stabilized older soil organic carbon under elevated CO₂, movement of newly fixed carbon from roots to stabilized soil organic carbon pools was found to be retarded along due to increased root biomass with concomitant decrease in new carbon in mineral-bound pools under elevated CO₂ (Cardon *et al.*, 2001). Changes in the below-ground food web structure may have long-term implications for litter

decomposition, increasing root respiration and rising inputs of litter and root exudates into soil under elevated CO₂ together with decreasing soil aeration due to higher water contents may cause increasing partial pressures of CO₂ in soil air (Bader and Körner, 2010; Bernhardt *et al.*, 2006). According to Henry's law, rising partial pressures of soil air CO₂ would increase concentration of carbonic acid (H₂CO₃) in soil solution, accelerating the soil acidification and mineral weathering (Oh and Richter, 2004) and increasing concentrations of inorganic carbon in soil leachate (Karberg *et al.*, 2005). But the increasing level of atmospheric CO₂ cannot directly affect soil mineralization processes because the soil CO₂ concentration is several orders of higher magnitude (Blagodatsky *et al.*, 2011).

2.6 Nodulation properties

Nitrogen fixation process in the leguminous plants mainly depends on their photosynthesis. Apart from photosynthesis, carbon dioxide and light separately affect nodulation and nitrogen fixation in leguminous plants (Hosoda *et al.*, 1978). As a strong sinks of carbohydrates (Walsh *et al.*, 1987), and nodules along with their root association could require up to 60% of photosynthetic assimilates produced over 12h photoperiod (Gordon *et al.*, 1997). So, the respiratory rate of a nodulated root system may be an order of magnitude greater than that of an equivalent non-nodulated root system (Vessey *et al.*, 1988). The absence of root nodules should accentuate the potential for carbohydrate feedback under elevated CO₂ (Arnone and Gordon, 1990). Nodule protein content, leghaemoglobin levels and enzyme activities was found to reach its peak value at approximately 50 days after taking stolon cuttings in white clover, and then declined to about 50% by 80 days (Gordon and James, 1997). In soybean or lupin nodules, nitrogenase activity increased as partial pressure of O₂ (pO₂) increased to some extent, but decreased when pO₂ exceeded the limit (Bergersen, 1962; Trinik *et al.*, 1976). Since legumes form a symbiotic association with N₂-fixing bacteria, they act as an extra sink for additional C for exchange with the bacterial symbiont to enhance N fixation (Udvardi and Day, 1997).

Variation in CO₂ concentration was reported to have an effect on growth of *Rhizobium* isolated from nodules (Lowe and Evans, 1962). Masterson and Sherwood (1978) reported about the CO₂ mediated decrease in reduced nodule weight per plant and nodule density (weight of nodules per gram of root) with increase in specific

nodule activity (acetylene reduction per plant and per gram of nodule) in pea and white clover. Finn and Brun (1982) reported no increase in specific nodule activity but total nodule activity increased with 56% more nodule dry weight in response to CO₂ enrichment for 16 days. The growth response of trees to elevated CO₂ was dependent on the availability of nitrogen, the actual mechanism through which elevated CO₂ influences the rate of N₂-fixation of field grown nodulated trees is still not clear (Temperton *et al.*, 2003a). Legumes' responsiveness to CO₂ enhancement was also found to be dependent on other environmental conditions (such as, water availability, temperature, etc.) (Serraj *et al.*, 1999; West *et al.*, 2005). Under water deficiency, it was reported that the initial decrease in nodule respiration resulted from changes in cortical respiration along with reduced nitrogenase activity (Serraj and Sinclair, 1996). Symbiotic N₂ fixation is an important source of N in many terrestrial ecosystems (Boring *et al.*, 1988, Ibewiro *et al.*, 2000), and the close coupling between C assimilation and symbiotic N₂ fixation suggests that N₂ fixation should increase due to increase in atmospheric CO₂ (Schortemeyer *et al.*, 2002; Gamper *et al.*, 2005).

Increases in growth and N₂ fixation in elevated CO₂ are well documented in annual legume species (Zanetti and Hartwig 1997; Dakora and Drake, 2000; Cabrerizo *et al.*, 2001; Schortemeyer *et al.*, 2002). Elevated CO₂ had been shown to stimulate the productivity of symbiotic N₂ fixing plants (Norby, 1987; Thomas *et al.*, 1991; Tissue *et al.*, 1997; Hungate *et al.*, 1999). In contrast, Arnone (1999) found no detectable stimulation of N₂ fixation under atmospheric CO₂ enrichment. Vogel *et al.* (1997) estimated that the elevated CO₂ grown plants fixed approximately 40% more N than ambient CO₂ grown plants. Increased root exudation of chemoattractants was involved in establishing the *Rhizobium* symbiosis, with an improved carbon supply to the nodules (Cabrerizo *et al.*, 2001), which were reported to be the main reasons for the stimulation of symbiotic N₂ fixation under elevated CO₂ (Marilley *et al.*, 1999). However, under soil P deficiency, N₂ fixation process was found to be strongly inhibited (Israel, 1987) with increase in specific N₂ fixation, thus partially compensating the poor nodulation effect to some extent (Almeida *et al.*, 2000) and reducing the response of N₂-fixing plants to CO₂ enrichment (Duchain *et al.*, 1993; Lam *et al.*, 2012b). N₂-fixing trees also were found to have the capacity to increase both C assimilation and total N₂ fixation with increasing atmospheric CO₂ (Thomas *et al.*, 2000) and this has been attributed to a greater mass and activity of root nodules,

leading to greater nitrogenase activity per plant (Whiting *et al.*, 1986; Norby 1987; Arnone and Gordon, 1990; Crush, 1993; Tissue *et al.*, 1997; Vogel *et al.*, 1997; Olesniewicz and Thomas 1999; Thomas *et al.*, 2000; Temperton *et al.*, 2003a) with very little overall effect on nodule relative efficiency (Crush, 1993). This ultimately modifies the soil N availability, ameliorating nitrogen limitations to ecosystem C storage under elevated CO₂ conditions (Schimel, 1995). Nodule number, biomass and the proportion of leghaemoglobin producing nodules were enhanced under elevated CO₂ (Arnone and Gordon, 1990; Hasse *et al.*, 2007) with enhancement in N₂ fixation in black locust (*Robinia pseudoacacia*) seedlings and 50% reduction in total plant P concentration under elevated CO₂ (Olesniewicz and Thomas, 1999). Root nodules of *Alnus glutinosa* (L.) exposed to three year elevated CO₂ produced a smaller and more evenly spaced root nodules than trees exposed to ambient CO₂ (Temperton *et al.*, 2003b). Schortemeyer *et al.* (1999) found no change in amount of nitrogen fixed per unit nodule dry mass under either ambient or elevated CO₂ in *Acacia*. West *et al.* (2005) documented the specificity of response of the legume N₂ fixation towards elevated CO₂ environment, depending on legume species and N supply. According to Erice *et al.* (2006a) elevated CO₂ did not affect the belowground nodule dry matter and the additional carbohydrate produced under elevated CO₂ may have been used, to increase specific nodule N-fixing activity. Existence of a positive feedback loop between nitrogen fixation and photosynthesis was observed in nodulated plants growing at elevated CO₂ (Arnone and Gordon, 1990). Legumes with their capability of fixing atmospheric N₂, would have an advantage in plant growth over non-N₂-fixers, showing a larger stimulation of growth and photosynthetic rate to elevated CO₂ (Ainsworth and Rogers, 2007). The greater photosynthetic rate in legumes grown under high CO₂ conditions would imply that there is a larger supply of organic C to nodules and this is used in turn by the bacteroid nitrogenase enzyme inside the nodules as a source of energy and reducing power to fix N₂ (Cabrerizo *et al.*, 2001). Symbiotic nitrogen fixation could also counterbalance the elevated CO₂-induced limitation of N availability in the rhizosphere and due to this reason leguminous plants played a key role in the response of ecosystems to elevated CO₂ (Marilley *et al.*, 1999). Zanetti *et al.* (1996) described the stimulation of symbiotic N₂ fixation in *Trifolium repens* under elevated atmospheric CO₂ in a grassland ecosystem, as a crucial factor in maintaining the C:N ratio in the ecosystem and accordingly, the

relative contribution of symbiotically fixed N_2 to total plant nitrogen increased under elevated CO_2 (Zanetti *et al.*, 1996; Schortemeyer *et al.*, 2002; Feng *et al.*, 2004). Feng *et al.* (2004) concluded that the enhanced growth in elevated CO_2 might not be restricted by N limitations as increased C uptake in response to increased CO_2 effect was followed by increased N_2 fixation. Under elevated CO_2 environment, increase in N yield reflects a stimulation of symbiotic N_2 fixation that was the result of the formation of more nodules rather than of higher specific N_2 fixation (Zanetti *et al.*, 1998). Under drought conditions in CO_2 enriched environment, nodulation and nodule activity were not regulated by carbohydrate availability but by a feedback mechanism mediated by the plant's demand for nitrogen (Serraj *et al.*, 1999). With increase in the end product of N_2 fixation activity (ammonia) (Aranjuelo *et al.*, 2009b) or decrease in shoot N demand, the concentration of N-transporting solutes declines with a consequent accumulation of products associated with the N_2 fixation (ureides) in the nodules that leads to inhibition of nitrogenase activity in the bacteroids (Serraj *et al.*, 1999). Continuous CO_2 enrichment led to increase the carbon-metabolising enzymes activity without increase in specific nitrogen fixation (Cabrerizo *et al.*, 2001). Hydroponics study revealed the enhancement in nodule CO_2 fixation under sufficient CO_2 application to roots and nodules, improving the nitrogen fixation and nodule formation in CO_2 enriched environment (Fischinger *et al.*, 2010).

2.7 Crop yield response to elevated CO_2

Ainsworth and Long (2005), reviewed ~31% increase in the light-saturated leaf photosynthetic rate with elevated CO_2 exposure to plant canopy due to 28% increase in the diurnal photosynthetic carbon assimilation. Elevated CO_2 was found to have a consistent fertilization effect on plant aboveground biomass as well as grain yield, thus modifying the harvest index (ratio of economic grain yield to the total dry matter yield at harvest), especially for C_3 plants (Kimball *et al.*, 2002). In spite of increase in total dry matter yield of sweet potato (Bhattacharya *et al.*, 1985), sugarbeet (Manderscheid *et al.*, 2010), potato tubers (Högy and Fangmeier, 2009a) due to greater partitioning of biomass into tubers than into roots with variable levels of atmospheric CO_2 enrichment, Högy and Fangmeier (2009a) reported a negative relationship between aboveground stem biomass of potato at canopy maturity and the level of atmospheric CO_2 enrichment. Kim *et al.* (2007), from their sunlit controlled

environmental chamber study reported about no effect of CO₂ enrichment on biomass accumulation in maize due to the effects of potential leaf warming under elevated CO₂ nullifying the elevated CO₂ induced stimulating effect on canopy photosynthesis, growth, and biomass accumulation. Reports are available for barnyard grass (*Echinochloa crusgalli* L.) with more tiller number and higher LAI under elevated CO₂ in rice field due to relative increase in the competitive ability of rice resulting from higher Net Assimilation Rate (NAR), with reduction for the same in barnyard grass due to inhibitory effect of elevated CO₂ (Zeng *et al.*, 2011). Elevated CO₂ was found to be the major stimulating factor for significant increase in aboveground plant biomass for berseem (Pal *et al.*, 2004), radish (Usuda, 2006), wheat (Porteaues *et al.*, 2009; Högy *et al.*, 2010a; Tausz-Posch *et al.*, 2012; Lam *et al.*, 2012a), barley (Wall *et al.*, 2011), *Brassica napus* L. (Frenck *et al.*, 2011; Franzaring *et al.*, 2011), rice (Lieffering *et al.*, 2004; Zeng *et al.*, 2011), pigeon pea (Saha *et al.*, 2011), soybean (Rogers *et al.*, 1986; Lam *et al.*, 2012b), due to the increased partitioning of photosynthetically assimilated carbon towards the growing organs of plants.

Reports are available about the stimulative effect of elevated CO₂ on grain yield of rice (Kim *et al.*, 2003; Lieffering *et al.*, 2004; Baker and Allen, 2005; Liu *et al.*, 2008) wheat (Wu *et al.*, 2004; Weigel and Manderscheid, 2012 : Lam *et al.*, 2012a), soybean (Cure *et al.*, 1988; Ziska *et al.*, 2001) pigeon pea (Vanaja *et al.*, 2010) potato (Craigon *et al.*, 2002; Högy and Fangmeier, 2009a), barley and sugar beet (Weigel, and Manderscheid, 2012). Grain yield was reported to remain unaltered for wheat and barley (Sæbø and Mortensen, 1996), soybean (Rogers *et al.*, 1986; Ferris *et al.*, 1999), with varying levels of atmospheric CO₂ enrichment.

Elevated CO₂ was found to increase wheat seed yield significantly due to increased tiller number and seed mass, but with decreased number of seeds per head resulting from a sustained increase in canopy quantum yield (Monje and Bugbee, 1998). Wu *et al.* (2004) found an increase in yield of wheat under elevated CO₂ treatment, mainly due to an increase in the number of wheat grains per plant. Li *et al.* (2000) reasoned that the increase in wheat grain weight under 550 µmol mol⁻¹ level of elevated CO₂ was due to faster rate of grain filling. Bunce (2008) reported that the change in the number of pods produced by *Phaseolus vulgaris* L, grown under elevated CO₂, was the primary determinant of the response of harvested seed yield instead of variation in photosynthetic response. δ¹³ C study revealed no significant

effect of elevated CO₂ on wheat biomass production and grain filling (Aranjuelo *et al.*, 2011). Cultivar specificity of the enhanced seed yield factor was revealed for soybean (Ziska *et al.*, 2001), spring wheat (Manderscheid and Weigel, 1997) and *Brassica napus* L. (Frenck *et al.*, 2011).

This clear cut trend of increase in crop dry matter accumulation under elevated CO₂ may not always results in decrease of harvest index value especially for C₃ crops, due to inconsistent behavior of different crop species towards economic grain yield under atmospheric CO₂ enrichment. The conversion ability of accumulated dry matter into the economic seed yield differs from one crop species to other with a large variable and erratic response of crop harvest index values (Cure *et al.*, 1986) under elevated CO₂ enrichment. Cure *et al.* (1986) reviewed decreasing effect of elevated CO₂ on soybean harvest index. Imai *et al.* (1985) found an increase in rice harvest index with 700 $\mu\text{mol mol}^{-1}$ CO₂ due to extension of ripening period by about six days. Significant increase of harvest index was noted for rice cultivar IR-36 only at elevated CO₂ level of 660 microbars in phytotron experiment (Ziska and Teramura, 1992). Atmospheric CO₂ enrichment levels of 580 and 680 $\mu\text{mol mol}^{-1}$, were found to cause a decline in harvest index for rice (Baker *et al.*, 1992; Ziska *et al.*, 1997; Kim *et al.*, 2001), mostly controlled by the number of grains and their growth rate, along with a significant reduction in the number of productive tillers relative to total tiller number under elevated CO₂ level of $\sim 580 \mu\text{mol mol}^{-1}$ (Kim *et al.*, 2003). In contrast, De costa *et al.* (2006) reported no effect of elevated CO₂ on rice harvest index, along with significantly greater percentage of filled grains under elevated CO₂. Increasing trend of harvest index, resulting from more partitioning of photosynthetic assimilates along with greater grain growth and seed yield was reported for wheat under 684 $\mu\text{mol mol}^{-1}$ level of CO₂ enrichment (Wheeler *et al.*, 1996). In contrast, slightly declining trend (not statistically significant) was found by Manderscheid *et al.* (2009) for wheat harvest index under elevated CO₂ levels of 660 and 550 $\mu\text{mol mol}^{-1}$. No influence of elevated CO₂ levels of 689 and 1200 $\mu\text{mol mol}^{-1}$ was also reported for wheat harvest index (Manderscheid and Weigel, 1997; Monje and Bugbee, 1998).

Harvest Index of broad bean (*Vicia faba*) was reported to increase under elevated CO₂ level of 700 $\mu\text{mol mol}^{-1}$ suggesting the natural evolutionarily advantage of annual plants to make full use of extra carbon assimilates to promote fitness by allocating the carbon assimilates (Wu and Wang, 2000). Harvest index of pigeon pea

was found to decline in CO₂ enriched environment of 580 $\mu\text{mol mol}^{-1}$, irrespective of cultivar specificity, due to lack of plant's capacity to utilize completely the more abundant photoassimilates under elevated CO₂ condition (Saha *et al.*, 2012). Chakraborty and Upreti (2012) reported an increase in harvest index in *B. juncea* and *B. campestris* for atmospheric CO₂ enrichment level of 550 $\pm$ 50 $\mu\text{mol mol}^{-1}$. Hasegawa *et al.* (2013) reported significant CO₂ $\times$ cultivar interaction of brown rice yield suggesting higher productivity under elevated CO₂.

Available literature explains the effect of elevated CO₂ on radiation utilization pattern of several crops, mostly for cereals, oilseeds and closed forest canopy. But for field grown pulse, it is scarcely reported one. Pulse crop constitutes a major share of our daily nutritional requirement. Therefore, extensive field scale research is essentially needed to evaluate the carbon capture and conversion efficiency for pulses.

3. MATERIALS AND METHODS

In order to achieve the objectives set out in the study, two years experiments on chickpea (*Cicer arittinum* L.) were conducted during *rabi* season (November-April) of 2010-11 and 2011-12. Details of the experiment, the methods followed and techniques adopted are described below:

3.1 Site description

The experiment were conducted in open-top chambers (OTCs) on chickpea (cv. Pusa 1105- *kabuli* type), during *rabi* season (mid November- mid April) of 2010-11 and 2011-12, at the farm of Indian Agricultural Research Institute, New Delhi, located at 28°35' N latitude, 77°12' E longitude and at an altitude of 228.16 m above mean sea level (MSL).

3.2 Climate and soil

The climate of the site is typical semi-arid with dry hot summer and mild winters with an average annual rainfall of 710 mm, of which 80 per cent is received during southwest monsoon from July to September. The mean daily maximum and minimum air temperature were between 10-29 °C and 2-12 °C, respectively during the crop season. The mean daily evaporation (USWB open pan evaporation) was 1.8 mm/day during the crop growth period.

The surface soil (0-30 cm) of the experimental site was non-calcareous and sandy loam in texture. The average bulk density of the soil is 1.61 Mg m⁻³; Water holding capacity 32.6% (v/v); saturated hydraulic conductivity 1.17 cm h⁻¹; pH (1:2.5 soil/water suspension) 7.6; EC 0.46 dS m⁻¹; organic C 0.03 g kg⁻¹; total N 0.031%; and available P and K, 5.046 and 453.86 kg ha⁻¹, respectively.

3.3 Experimental details and experimental design

The circular structure of OTCs was fabricated using aluminum frame and fixed in the field as described by Saha *et al.* (2011). The diameter and height of each OTC was 3.0 and 2.5 m respectively. The frame was lined with polyvinyl chloride (PVC) sheets

(120 μm thickness), which transmitted 85% of natural sunlight. Upper part of OTCs had a frustum of 0.5 m at 2.5 m height, to reduce the dilution of CO_2 by air current inside the chambers.

Pure CO_2 gas (99.7%, v/v CO_2 and less than $10 \mu\text{mol mol}^{-1}$ CO) was released from a commercial grade cylinder fitted with a regulator (DURA, make ESAB, India) through solenoid valve and PV tubing connected to air-exhaust blower mounted at the base of each OTC. To maintain desired elevated levels of CO_2 in two OTCs at $580 \mu\text{mol mol}^{-1}$, continuous pure CO_2 was injected into the hollow ring of the OTC, where it was mixed with air from an air compressor before entering into the chamber. The air sample from the middle of the chamber was drawn periodically into a CO_2 sensor (NDIR, make Topak, USA) to monitor CO_2 concentration. The set level of CO_2 was maintained with the help of solenoid valves which were controlled by Program Logic Control (PLC) and Supervisory Control and Data Acquisition (SCADA) system running Winlog software (Make SELCO, Italy).

During the entire period of experiment, temperature and humidity inside all the four chambers were continuously recorded with the help of automatic sensors (Model TRH 511, Ambetronics, Switzerland) fitted in the middle of each OTC and connected to data logger (Model TC 800D, Ambetronics, Switzerland). In two OTCs, elevated CO_2 levels of $580 \mu\text{mol mol}^{-1}$ were maintained from 6:00 AM to 6:00 PM from the crop emergence and the other two OTCs were used as chamber controls without any external supply of CO_2 . Regular washing of the OTC chambers was done using a gentle stream of water to remove the dust and foreign material and to maintain PVC sheet transparency.

3.4 Crop Management practices

Chickpea seeds were treated with the fungicide captan @ 2 g kg^{-1} of seed. On the next day, seeds were surface sterilized by dipping in 0.1 % HgCl_2 for 2 min. After rinsing in sterile distilled water, seeds were allowed to imbibe water for 6 hr and were then coated with *Rhizobium* culture and 10% sucrose solution in shade. The seeds were then sown manually at 10 cm depth by putting 2–3 seed per hole in rows drawn by tyne by maintaining a row-to-row spacing of 30 cm and plant-to-plant spacing of 15 cm. Farmyard manure was applied @ 2.5 t ha^{-1} at 15 days before sowing of the crop.

Basal dose of 20-40-60 NPK was incorporated during seed bed preparation and the sowing operation was completed within second week of November. After 3 weeks, plants were thinned and allowed to grow with a density of 20 plant/ m² in each open OTC. Weeding was done manually, while fungicide mixture (Bavistin + Dithane M-45) was sprayed at 50 days after sowing (DAS) to prevent fungal infection.

3.5 Radiation Characteristics

Canopy radiation extinction coefficient (k) and Radiation Use Efficiency (RUE)

Intercepted Photosynthetically Active Radiation (IPAR) by the whole canopy was calculated by subtracting outgoing radiation at the bottom of the canopy (I) from the incident radiation on the top of the canopy (I₀). Both incoming and outgoing PAR values were measured weekly in $\mu\text{mol m}^{-2} \text{s}^{-1}$ unit, at the top and bottom of chickpea crop throughout the season using Apogee point quantum sensor (SQ-215 model). Eight measurements were averaged for each chamber, four with inter-row placement and the other four with intra-row placement. To get reflected radiation from top and bottom of the canopy, the sensor was held in inverse position. The above measurements were taken on clear days between 11:30 and 12:30 hours IST when disturbances due to leaf shading, leaf curling and solar angle was minimum. From the readings of IPAR, the fractional interception of PAR (fIPAR) was calculated as

$$\text{fIPAR} = (I_0 - I) / I_0 \quad \dots\dots\dots(1)$$

Canopy radiation extinction coefficient (k) was calculated as least-square regression slope of the relationship between $\ln(1 - \text{fIPAR})$ and LAI (Robertson *et al.*, 2001). It indicates the effectiveness of a crop canopy at intercepting PAR (Charles- Edwards *et al.*, 1986). Values of fIPAR for each day after sowing were interpolated between actual measurements using fitted polynomial curves. The canopy fIPAR and LAI were related using following equation:

$$\text{fIPAR} = 1 - \exp(-k \times \text{LAI}) \quad \dots\dots\dots(2)$$

Cumulative intercepted radiation was calculated as product of daily incident global radiation measured by pyranometer and fIPAR of each day after sowing (DAS), with making the correction for 85% transmittance of sunlight through the polythene cover

of OTCs. The Radiation-use efficiency (RUE) has been defined as the ratio of biomass production to the amount of solar radiation intercepted for a wide range of crops at canopy level for week to monthly time scales (Monteith, 1977) RUE is characterized by the linear relationship between total biomass production and cumulative intercepted radiation. The RUE was calculated as slope of the relationship between cumulative IPAR and biomass, using least square regression (Saha *et al.*, 2012).

3.6 Canopy thermal properties

A hand-held infrared thermometer (AG-42, Telatemp Crop. USA) with 8° field of view, was used to measure canopy temperature (°C). The value for each chamber were the mean of four readings, which was taken from third row of each side of OTC, at an angle of approximately 45° to the horizontal, in a range of directions such that it shot plant canopy. Special care was taken for the thermometer not to view areas other than plant canopy or leaves. Data were recorded around midday at regular interval during crop growth period. At the time of canopy temperature measurement, air temperature and corresponding canopy air temperature difference (CATD) for each OTC was also recorded.

3.6 Crop Growth and physiological parameters

3.6.1 Leaf Area Index (LAI)

Leaf Area Index is defined as half of the total leaf area (i.e., the area for only one leaf surface) divided by the ground surface area beneath those leaves (e.g., Chen and Black, 1991; Jonckheere *et al.*, 2004). Leaf-area index was measured at weekly interval with a plant canopy analyzer (LAI-2000, Li-COR, Lincoln, NE, USA), which is based on ‘fish-eye’ measurement of diffuse radiation interception by measuring gap fraction at five zenith angles (0-13, 16-28, 32-43, 47-58, 61-74°) simultaneously. The measured gap-fractions are inverted to obtain the effective LAI and the instrument was set to take three below and one above canopy measurements to estimate the LAI. The LAI was measured on cloudy days or within 1 h of sunset when the incident sunlight was diffuse. It was measured at four random locations within each OTC and these locations spanned an area of the plot reserved for LAI and protected from

destructive sampling. A quarter view cap of 45° was used to exclude the operator, and the rest of hemispherical view was used to measure LAI.

3.6.2 Transpiration rate, leaf temperature and stomatal resistance

The LI-1600 Steady State Porometer (LICOR Inc., Lincoln NE, USA) was used for rapid and direct readout of stomatal resistance, concurrent measurements of relative humidity (RH %) of cuvette, leaf temperature and transpiration rate. Water loss from a leaf placed in the LI-1600 cuvette was determined by measuring the flow rate of dry air needed to maintain a constant RH inside the cuvette. Typically, ambient RH was used as a null-point and dry air was injected into the cuvette at a rate that was sufficient to balance the transpiration water flux out of the leaf. This maintained the cuvette RH at a set point. Stomatal resistance was calculated directly from the measured values of RH, temperature of the leaf and air, and flow rate. It was expressed in terms of % change of diffusive resistance (ΔR) and the magnitude of changed leaf temperature (ΔT) in °C under elevated CO₂ over ambient CO₂ treated plant leaf. Measurements were made at prevailing light and climatic conditions between 10:00 h and 13:00 h periodically during the field experiment. Measurements were taken on four randomly selected physiologically matured leaves at the top of the canopy for each genotype and chamber. Abaxial leaf surface were used due to easy handling. For the serrate leaflet of chick pea, the fully expanded second and third leaf was combined to make a uniform leaf layer for placing in the cuvette of LICOR-1600.

3.7 Plant sampling at different crop growth stages

To estimate the dry matter partitioning and to calculate the resource use efficiency (radiation use efficiency and water use efficiency), above ground plant parts were harvested randomly in triplicate from each OTC at three distinct crop growth stages, viz. initial vegetative, 50% flowering and pod maturity stage. Based on physical appearance, the specific crop growth stages appeared at 50, 94 and 125 DAS for 2010-11 and 48, 72 and 90 DAS for 2011-12, respectively.

The harvested plants were washed and the leaves were separated after recording of the morphological characters (plant height and fresh biomass production). The same plant samples were used for measurement of dry biomass and

the leaf area index. The green leaf portions of the cut plants were separated and the total leaf area per plant was measured using leaf area meter (LICOR-100) and LAI was also calculated as:

$$\text{LAI} = \frac{\text{Measured leaf area per plant (cm}^2\text{)} \times \text{Number of plants per m}^2}{100 \times 100 \text{ (cm}^2\text{)}} \quad \dots\dots\dots (3)$$

3.7.1 Specific Leaf Area and Specific Leaf Nitrogen

To derive the specific leaf area (SLA; leaf area per leaf dry matter in $\text{m}^2 \text{g}^{-1}$), leaves were separated from other plant parts and the total leaf area per plant was measured using leaf area meter (LICOR-100). The leaves were then oven-dried at 65°C for 48 hours and weighed precisely (up to 4 digit) by a digital balance. The dried leaf samples from each biomass harvest were then ground, mixed, and milled to a fine powder (1 mm). A sub sample of 100mg was withdrawn and analysed for leaf nitrogen content using micro-Kjeldahl digestion as described below. Specific leaf nitrogen (SLN) in g N m^{-2} per unit leaf area was calculated as the quotient of leaf nitrogen content and specific leaf area (Muchow, 1988).

3.7.2 Leaf chlorophyll determination

Fresh leaf samples from different treatments were cut and 100 mg of it was weighed accurately with an analytical balance and chlorophyll was extracted by a non macerated method by equilibrating it with 10 ml DMSO (Dimethyl Sulphoxide) in a capped vial and keeping in an oven at 65°C for about 3 hours (Hiscox and Israelstam, 1979). The decanted solution was used to estimate the absorbance at 645 and 663 nm wavelength using Spectronic-20 Spectrophotometer. For carotinoids content, absorbance was taken at 480 nm wavelength and derived from the equations proposed by Kirk and Allen (1965). The leaf chlorophyll content was calculated using the modified form of the formula given by Arnon (1949). Chlorophyll and carotinoids content was expressed in leaf weight basis (mg of Chl / gm of leaf fresh weight).

$$\text{Total Chlorophyll} = (8.02A_{645} + 20.2A_{663}) (V/1000 \times W) \quad \dots\dots\dots (4)$$

$$\text{Chlorophyll a} = (12.7A_{663} - 2.69A_{645}) (V/1000 \times W) \quad \dots\dots\dots (5)$$

$$\text{Chlorophyll b} = (22.9A_{645} - 4.68A_{663}) (V/1000 \times W) \quad \dots\dots\dots (6)$$

$$\text{Total carotinoids} = (A_{480} + 0.114A_{645} - 0.638A_{663}) (V/1000 \times W) \quad \dots\dots\dots (7)$$

Where, V is the final volume (ml) of extract, W is the weight fresh leaf sample and A_{480} , A_{645} and A_{663} are the absorbance at 480, 645 and 663 nm, respectively.

3.7.3 Relative Leaf Water Content (RLWC)

Relative leaf water content was calculated according to the method given by Barr and Weatherley (1962). For relative leaf water content measurement, the second leaf from top of the plant (three leaves from each plot) was taken carefully without any damage. Then fresh weight of the leaf was taken as quickly as possible to avoid any moisture loss after cutting the leaf into pieces. Thereafter, water was added to saturate the leaf pieces and kept for 24 hours for attaining the equilibrium. Then saturated leaf weight was recorded and were driedt in the oven at 65⁰C. At last, dry leaf weight was recorded. The RLWC was recorded at each of the three stages of destructive sampling during both of the years. The relative leaf water content was calculated by the following formula:

$$\text{Relative leaf water content} = \frac{F - D}{S - D} \quad \dots\dots\dots(8)$$

where, F = weight of fresh leaf (mg)

S = weight of saturated leaf (mg)

D = weight of dry leaf (mg)

3.7.4 Leaf water potential (ψ)

Second leaf from top of the plants (three leaves from each OTC) was sampled with intact petiol between 10:00-11:00 AM and brought to the laboratory in enclosed polythene bags protected from direct sunlight. Leaf water potential (ψ) was measured using a pressure chamber (s-pms Instruments, New Delhi) following the method of Scholander *et al.* (1964). Each value was mean of four plants from each OTC. Prior to this measurement, the wall of the chamber was lined with moist filter paper and the petiole of an excised leaf was sealed into the chamber such that its cut basal end projected outside (atmospheric pressure) and the axial or leaf blade surface remained inside where it was subjected to controlled gas pressure. When the sample was excised, xylem sap pressure potential was increased to atmospheric pressure and sap withdraws from the cut surface in response to its movement from xylem into

surrounding cells. When the increasing gas pressure was applied on the blade inside the chamber, cell sap water potential correspondingly increased forcing water back into the xylem. When surface of the xylem sap just returned to the cut surface, the applied pressure equals to that of the sap pressure in the xylem vessels. This balancing pressure (in bar) which was equal to the leaf water potential was recorded. The ψ were measured on the same days after sowing as that of RLWC.

3.7.5 Water soluble leaf carbohydrate analysis

For analysis of water soluble carbohydrate content, 100 mg dry leaf sub sample was taken and shaken in 10 ml hot water for 30 min at 80°C in a water bath. After filtration, the concentration of carbohydrates in the aqueous solution was determined by the anthrone method (Kruse *et al.*, 2008). 1 ml aliquot was taken in four replicate and 4 ml of anthrone reagent was added with eight minutes of heating in a boiling water bath. Then cooled rapidly and the intensity of dark green color was read at 630 nm against a blank in UV- VIS spectrophotometer.

3.7.6 Determination of carbon content of different plant parts

Homogenate dried roots and stem portion of chickpea plants and ground chickpea flour were oven dried at 80°C for 24 hours. Ash (%) of the plant sample was determined by placing accurately weighed 1g of well ground sub sample into a porcelain crucible and ignited at about 500°C for 6 hours in muffle furnace and cooled in desiccator as quickly as possible to prevent the moisture absorption. After cooling to room temperature, it was reweighed by a precise digital balance (Piper, 1966). Carbon concentration of leaf biomass was estimated after Poincelot (1972) and C:N ratio was calculated from the obtained results. Ash content and mass loss-on ignition (LOI) was calculated by the formula:

$$\text{Ash content (\% as fed basis)} = (\text{weight of residual ash} \times 100) / \text{weight of sample} \quad \dots\dots\dots (9)$$

$$\text{Ash content (\% adjusting to dry basis)} = (\text{Ash content (\% as fed basis} \times 100) / \text{Dry matter \% as on fed basis} \quad \dots\dots\dots (10)$$

Where Dry matter % = (weight of dry sample at 105°C×100) / weight of samples before drying (11)

Mass loss on ignition (%) = (1- % Ash content) (12)

Carbon % in plant leaf = (100-ash %) /1.8 (13)

3.7.6.1 Total Organic Carbon

High temperature catalytic oxidation at 950°C and simultaneous CO₂-measurement in both soil and grain samples were performed by Elementar Analysen systeme GmbH: vario TOC cube.

3.7.7 Root analysis

3.7.7.1 Root sampling

Four root samples from each OTC were collected at three crop growth stages in both the years. After removing the above-ground plant parts, the soil surface was cleaned. The core of the auger was placed so as to keep the base of the stem at the centre and then soil core was excavated from each 15 cm depth layer down to a 45 cm depth. After the excavation, the soil cores were sealed in polythene bags and stored in cold room (maintained at 4°C) for later scanning.

3.7.7.2 Scanning and Image analysis using WINRHIZO system

Scanning and image analysis for root characteristics was carried out using Root Scanner (LA-1600) and the root morphology and architecture measurements (total root length, root surface area, root thickness and root volume) were done by win-RHIZO program (Regent Instruments Inc. Canada). The cleaned root samples were dried in an oven at 65⁰C for 48 hours, and weighed. The root dry weight, root length, surface area and average diameter measured were divided by the volume of the soil core, from which the respective densities were calculated for each depth of soil.

3.8 Spectral Measurements

Handheld spectroradiometer having 25° Field of View (FOV) was used for spectral reflectance measurement of chickpea in the OTC condition. FieldSpec TM3 spectroradiometer of Analytical Spectral Device (ASD) make recorded canopy

spectral reflectance between 350 and 2500nm wavelength at 1 nm interval. Spectral reflectance was derived as the ratio of reflected radiance to incident radiance which was estimated by using a calibrated white reference (Spectralon). The spectral data were processed and exported to Microsoft Excel through ASD ViewSpec Pro software. Thereafter, Normalized Difference Water Index was calculated as follows (Gao, 1996)

$$\text{Normalized Difference Water Index (NDWI)} = (R_{857} - R_{1640}) / (R_{857} + R_{1640}) \quad \dots\dots\dots(14)$$

where, R is reflectance at specified wavelength (nm).

3.9 Yield component analysis

The experiment was terminated at the harvesting maturity of the crop at 142 days and 112 days after sowing during 2010-11 and 2011-12, respectively, when the entire crop dried out with a yellowish brown appearance. Grain yield, final biomass and other yield parameters, like pod number, seed number, and number of seeds per pod were recorded on per plant basis. Leaves, stem and pods were separated and dried to constant weight at 70°C for around 48 hours in an oven and their dry weights were recorded by a precise digital balance.

3.10 Study of soil characteristics

3.10.1 Soil moisture (v/v) monitoring

For monitoring soil moisture, neutron probe access tube was permanently installed to a depth of 1.5 m in each of the four OTC at the centre. The soil moisture content was monitored at a counting rate of 60 seconds at weekly interval using the neutron moisture meter (CPN 503 DR HYDROPROBE MOISTURE GAUGE, International INC. USA.) for each 15-cm layer increments (15-30, 30-45, 45-60, 60-75, 75-90, 90-105 and 105-120 cm depth). Gravimetric soil moisture content was determined for surface soil (0-15 cm), periodically and at harvest by drying 10 g moist soil to constant mass at 105°C. All the measurements were carried out in triplicate. For preparation of calibration for NMM curve, the entire soil profile of all the OTCs were subjected to saturation once, during a long rain free period of hot summer months (May to July). Subsequent measurements of the depleting soil moisture content were

performed periodically at the specified depths by gravimetric method of soil moisture determination with a screw auger and the neutron probe was calibrated against soil water content determined gravimetrically and bulk density of the soil at the experimental site (Zhang *et al.*, 2000).

Soil moisture depletion rates were calculated in mm day^{-1} between two adjacent sampling dates. The cumulated depletion was calculated during entire crop growth period for both of the years. From the soil moisture depletion data of the entire crop growth period, the cumulated crop water use was computed using soil water balance equation and the root water uptake was computed for the specific crop growth stages up to maturity. To account for the deep drainage component, periodic changes in volumetric soil moisture content at lower depths (viz. 90-105 and 105-120 cm) was summed up and deducted from the gross cumulated soil moisture depletion for the entire crop growth period. Thus actual value of crop water use was obtained. Water use efficiency (WUE) of the crop was calculated from the regression between biomass (g) and cumulated crop water used (cm).

3.10.2 Laboratory analysis of soil samples

3.10.2.1 Collection, preparation and preservation of soil samples

For aggregation study, surface soil samples (0 to 30 cm) were collected by *khurpi* and soil microbial activity along with the soil enzyme assay study was carried out at the earliest with fresh field moist rhizosphere soils. Otherwise, all the soil samples were collected by core sampler from 0-30 cm depth at six random places in each OTC before the sowing of the crop and after final harvest and preserved in polythene bags at 10 °C till the analysis. The soil samples were air-dried in the laboratory at room temperature (~25 °C) to constant weight and passed through a 2 mm sieve to obtain a homogeneous soil sample material for further chemical analysis.

3.10.2.2 Soil physico-chemical properties

3.10.2.2.1 Mechanical composition of soil

The percent sand, silt and clay contents were determined by hydrometer method (Bouyoucos, 1962), and soil texture was determined with the help of texture triangle as proposed by USDA.

3.10.2.2.2 Bulk density (BD)

The core sampler was pushed into the soil to the desired depth in such a way that soil core is collected from the centre of the given depth (0-15 and 15-30 cm). Soil samples were dried in oven at 105°C for 48 hrs. Bulk density (Mg m^{-3}) was calculated by dividing weight of dried soil by the volume of core used (Veihmeyer and Hendrickson, 1948; Blake and Hartge, 1986). Bulk density of soil was calculated from the formula:

$$\text{BD} = (X - Y) / V \quad \dots\dots\dots (15)$$

where, X = weight of core with oven dry soil

Y = weight of empty core

V = volume of core

3.10.2.2.3 Saturated hydraulic conductivity

Measurement of the hydraulic conductivity of saturated soils in the laboratory was based on the direct application of the Darcy's equation to a saturated soil column of uniform cross sectional area (Klute and Dirksen, 1986). A hydraulic head of 2.45 cm was imposed on the soil column and the resulting, constant flux of water was measured. The saturated hydraulic conductivity was calculated by the equation.

$$K_s = \frac{V.L}{A.t(H_2-H_1)} \quad \dots\dots\dots (16)$$

where,

K_s = Saturated hydraulic conductivity (cm/hr)

V = Volume of water (cm^3) that flows through the sample of length L (cm) and cross sectional area A (cm^2) in time t

H_2-H_1 = Hydraulic head difference imposed across the sample of length L (cm)

3.10.2.2.4 Mean weight diameter (MWD)

Aggregate stability was measured by Le Bissonais method (Le Bissonais, 1996). At the end of the crop season, soil sample was collected in four replicates from surface, using 100 mm steel ring pushed into the soil to a depth of 50 mm. The sample was air dried and then forced through a 5 mm sieve and 3 – 5 mm aggregates selected for the tests. Just prior to the 3 treatments, aggregates were put into an oven at 40 °C for 24 h so they are at a constant matric potential.

Treatment 1 (Fast wetting)

5g of prepared soil aggregate sample was gently immersed in a 250 ml beaker filled with 50 ml deionised water for 10 min. The water was then sucked off with a pipette and the soil material was transferred to a 50 µm sieve previously immersed in methylated spirit for measurement of fragment size distribution

Treatment 2 (Slow wetting)

5g of prepared soil aggregate sample was placed on a filter paper on a tension table at a matric potential of –3 kPa. for 30 min and aggregates were transferred to a 50 µm sieve previously immersed in methylated spirit for the measurement of fragment size distribution.

Treatment 3 (Mechanical breakdown by shaking after pre-wetting)

5g of prepared soil aggregate sample was gently immersed in a 250 ml beaker filled with 50 ml of methylated spirit for 10 min and sucked off with a pipette. The soil material was then transferred to a 250 ml conical flask with 50 ml of de-ionised water. The water content was then adjusted to 200ml. The conical flask was corked and agitated by turning end over end 20 times and was left for 30 min for sedimentation of coarse fragments. Excess water was then sucked off with a pipette and the remaining mixture of soil and water was transferred to a 50µm sieve previously immersed in methylated spirit for the measurement of particle size distribution.

Fragment size distribution and measurement

A 50 µm sieve (previously immersed in methylated spirit) and containing the soil material from the previous treatments was gently moved up and down in the Yoder sieve apparatus. This separates fragments < 50 µm from those > 50 µm. Methylated

spirit was used for wet sieving because it reduces additional breakdown. The $> 50 \mu\text{m}$ fraction was collected from the $50 \mu\text{m}$ sieve and left to air-dry overnight and then oven dried overnight at 105°C . The soil material was then gently sieved by hand through a column of six sieves (2000, 1000, 500, 200, 100 and $50 \mu\text{m}$). The mass percentage in each size fraction was then calculated. The fraction, $50 \mu\text{m}$ is the difference between the initial mass and the sum of the six other fractions. The aggregate stability for each breakdown mechanism was expressed as fragment size distribution (FSD) in seven classes or the mean weight diameter (MWD), which is the sum of the mass fraction remaining in each sieve multiplied by the mean aperture of the adjacent mesh. MWD's ranged from $25 \mu\text{m}$ to 3.5 mm using the set of six sieves.

3.10.2.3 Soil reaction (pH) and electrical conductivity (EC)

Soil pH was determined in 1:2.5 (w/v) soil-water suspensions using combined electrode. The EC was determined in the supernatant of the same soil: water ratio by a conductivity bridge as described by Jackson (1973) and expressed in dSm^{-1} .

3.10.2.4 Dehydrogenase enzyme assay

Dehydrogenase activity of the moist rhizosphere soil was determined by observing the magnitude of reduction of triphenyl tetrazolium chloride (TTC) to triphenyl formazan (TPF). 6g of air dried soil ($<2 \text{ mm}$) was placed in screw-cap glass test tubes (three replicates samples per soil). 1 ml of 3% aqueous solution of TTC was added along with 2.5 ml of distilled water in each tube. After thorough mixing, the test tubes were capped and incubated at 37°C for 24 h in darkness. After 24 hours the stopper was removed and 10 ml methanol was added. The content of each test tube was mixed through vortexing and then filtered. The intensity of reddish color was recorded by UV-VIS spectrophotometer at 485 nm wavelength and the activity was expressed in μg of TPF produced g^{-1} of dry soil day^{-1} by using calibration graph prepared from TPF standards (Casida *et al.*, 1964).

3.10.2.5 FDA Hydrolysis activity in soil

The FDA hydrolysis assay was carried out by taking 1 g of rhizosphere soil in three replicates for each OTC and was suspended in potassium phosphate buffer (pH 7.6) and FDA (0.5 mg ml^{-1}). After incubation for 2 h at 37°C , the reaction was stopped by

adding acetone. The solution was filtered through Whatman No. 1 filter paper and the absorbance of the supernatant was measured at 490 nm by UV- VIS spectrophotometer using fluorescein standard and the values were represented as μg fluorescein released g^{-1} of soil h^{-1} (Green *et al.*, 2006).

3.10.2.6 Acid and alkaline phosphatase assay in soil

The acid and alkaline phosphatase activity of the soil were determined by following methods as described by Tabatabai and Bremner (1969) and was expressed in μg *p*-nitrophenol g^{-1} of dry soil hr^{-1} . For estimation of acid phosphatase activity, 1 g soil (<2 mm) was placed in 4 ml MUB buffer (pH 6.5 for acid phosphatase and pH 11 for alkaline phosphatase) and was incubated with 1 mM *p*-nitrophenyl phosphate in the same buffer at 37°C in water bath. After 1 hour the reaction was terminated by the addition of 1 ml of 0.5 M CaCl_2 and 4 ml of 0.5 M NaOH and the suspension was filtered through a Whatman no. 2v folded filter paper. The optical density was measured at 505 nm in a UV- VIS spectrophotometer. Controls were examined for each to allow for the color not derived from *p*-nitrophenol released by phosphates activity. To perform controls similar procedure was followed, but with the addition of 1 ml of *p*-nitrophenyl phosphate solution after the addition of 0.5M CaCl_2 and 4 ml of 0.5 M NaOH (i.e. immediately before the filtration of suspension).

3.10.2.7 β Glucosidase Assay in soil

β glucosidase is involved in the degradation of cellulose in soils. β -glucosidase activity was assayed by the method of Eivazi and Tabatabai (1988), using the substrate analogue *p*-nitrophenyl- β -D-glucopyranoside (*p*NPG). 1g of soil (<2 mm) was weighed into screw-cap glass test tubes (three replicate samples per soil) and incubated for 1h in a incubator at 37°C with 0.25 ml of toluene, 4 ml of 0.05 M modified universal buffer (pH 6.0) and 1 ml of 0.025 M *p*NPG dissolved in buffer. After 1 hour the reaction was terminated by adding 1 ml of 0.5M CaCl_2 and 4 ml of 0.2M *Tris*-hydroxymethyl (aminomethane) (THAM), adjusted to pH 12 with NaOH. The mixture was swirled and then centrifuged for 10 min at $1500 \times g$ and the absorbance was measured at 410 nm. Values were corrected for a blank (substrate added immediately after the addition of CaCl_2 and *Tris*-NaOH) and for adsorption of

released *p*-nitrophenol in the soil. β -glucosidase activity was expressed as $\mu\text{mol } p\text{-nitrophenol released g}^{-1} \text{ of soil h}^{-1}$.

3.10.2.8 Analysis of Nutrient pools

3.10.2.8.1 Total nitrogen content by Micro-Kjeldahl method

The Micro-Kjeldahl method is the most reliable method for nitrogen estimation in plant and soil samples. The nitrogen content was determined by digesting the samples in concentrated sulphuric acid at high temperature with catalyst to convert organic and inorganic forms of nitrogen into ammonium sulphate. This salt on steam distillation with excess of NaOH liberates ammonia, which was collected in boric acid solution. The ammonium borate formed was then titrated with standard sulphuric acid.

1g of soil (<2 mm) (0.1 g powdered leaf or grain sample or 0.2 g powdered nodule) sample was placed in digestion flask. 1.1 g of digestion mixture (K_2SO_4 , CuSO_4 and Selenium powder in the ratio of 100:10:1), 0.5 g sodium thiosulphate and 4 ml of sulphuric-salicylic acid mixture (25 g salicylic acid in 1 L H_2SO_4) were added. The sample was digested using the digestion unit by raising the temperature to 150 °C, 250 °C, 350 °C and 450 °C. Once the temperature reached 450 °C, again it was kept for half an hour. The digestion was carried out for 2 hours till a green clear solution was obtained and then cooled and 20 ml of distilled water was added to dissolve the solid. 100 ml conical flask containing 10 ml of boric acid was taken. 2 to 4 drops of mixed indicator dye (0.099 g Bromocresol green and 0.066 g of Methyl red in 100 ml of ethanol) was added and the flask was placed beneath the condenser with the delivery tip immersed in the solution. The digest was transferred to distillation apparatus unit keeping the outlet of the condenser tube dipped well in acid and the steam distilled until 20 ml of distillate was collected in the conical flask. The tip was rinsed with distilled water and the distillate was titrated against the 0.1N standard sulphuric acid solution until the first appearance of pink colour, as the end point. A blank was also run containing the same quantities of all the reagents but without sample for every set of nitrogen determination (Bremner, 1960).

Percent nitrogen in sample was calculated as follows:

$$\%N = \frac{(\text{ml } \text{H}_2\text{SO}_4 \text{ in Sample} - \text{ml } \text{H}_2\text{SO}_4 \text{ in blank}) \times \text{Normality of } \text{H}_2\text{SO}_4 \times 14 \times 100}{\text{Weight of sample in g}}$$

Mass of sample (mg)

3.10.2.8.2 Available phosphorus and potassium in soil

The soil samples were extracted with 0.5 M NaHCO₃; pH 8.5 (Olsen *et al.*, 1954). Phosphorus content in the extracts was determined by ascorbic acid method (Watanabe and Olsen, 1965). Ammonium acetate (pH 7.0) extractable K was determined by flame photometry (Hanway and Heidel, 1952).

3.10.2.9 Analysis of soil carbon pools

3.10.2.9.1 Total organic carbon (TOC)

The study used method given by Schollenberger (1927). First, 0.25 g soil (<2 mm) was taken in a centrifuge tube and it was washed twice with water to remove the excess salt and then it was taken in a 25 ml glass test tube. Then, 10 ml of 0.4 N chromic acid (with Ag₂SO₄ @ 1.25% to concentrated H₂SO₄) was added and placed on a 85% orthophosphoric acid bath at 155-160°C for 20-25 minutes. This content was mixed every 5 minutes and when the temperature was reached at 155°C (boiling starts) then it was kept for more 5 minutes. After that the test tube was removed from the bath and allowed to be cooled before transferring solution to a titration vessel with approximately 100 ml of distilled or deionized water. Then 5 ml of 85% H₃PO₄ and four drops of *o*-phenanthroline or diphenyl amine indicator were added to it. It was back titrated with Fe (NH₄)₂(SO₄)₂ solution (FAS) to a color change from green to reddish brown for *o*-phenanthroline and violet-blue to green for diphenylamine. Two unheated blanks were also titrated to standardize the Fe (NH₄)₂(SO₄)₂ solution. TOC was calculated as follows:

$$\text{meq of H}_2\text{Cr}_2\text{O}_7 = \text{ml} \times N = 10 \times 0.4 = 4 \quad \dots\dots\dots (17)$$

$$\text{Normality of FAS} = (\text{ml} \times N \text{ of H}_2\text{Cr}_2\text{O}_7 / \text{Blank}) = 4/\text{Blank} \quad \dots\dots\dots (18)$$

$$\text{meq of FAS} = \text{ml} \times N = \text{ml consumed in Sample} \times 4/\text{Blank} \quad \dots\dots\dots (19)$$

$$\text{TOC}(\%) = (\text{meq of H}_2\text{Cr}_2\text{O}_7 - \text{meq of FAS}) \times 0.003 \times 100/\text{wt of soil} \times 100/90 \quad \dots\dots\dots (20)$$

Here, 0.003 is conversion factor for carbon, 100/90 for 90 per cent recovery of this method and 100 to get result in per cent.

3.10.2.9.2 Oxidizable organic carbon and its fractions

The content of oxidizable organic carbon and its different fractions in the soil were estimated following procedure of Walkley and Black (1934) as modified by Chan *et al.* (2001)

Walkley and Black Carbon (WBC)

Organic carbon content of the soil samples (< 0.2 mm sieve) was determined following the wet digestion method (Walkley and Black, 1934; Walkley, 1947). 1 g soil was digested with 10 ml potassium dichromate ($K_2Cr_2O_7$) and 20 ml conc. H_2SO_4 in dark condition for 30 min. Then excess dichromate was determined by titration with ferrous ammonium sulphate [$Fe(NH_4)(SO_4)_2 \cdot 6H_2O$] after adding 10 ml conc. H_3PO_4 using Diphenyl amine indicator. Similarly, blank experiment was carried out to determine the amount of dichromate used for digestion of carbon which indirectly tells the amount of carbon in soil. Organic carbon percentage was calculated from the following formula:

$$\% \text{ Organic C (corrected)} = 1.3 * 0.5 * (B-S) * 0.003 * 100/3 \quad \dots\dots (21)$$

To determine different fractions of oxidizable organic carbon, the method proposed by Chan *et al.* (2001) was adapted using 5, 10 and 20 ml of concentrated (18.0 mol L^{-1}) H_2SO_4 and $K_2Cr_2O_7$ solution. This resulted in three acid-aqueous solution ratios of 0.5:1, 1:1 and 2:1 that corresponded to 6.0, 9.0 and 12.0 mol L^{-1} H_2SO_4 , respectively, and produced different amounts of heat of reaction to bring about oxidation of SOC of varying oxidizability. The amounts of oxidizable organic carbon thus determined allowed distribution of TOC into the following four fractions of decreasing oxidizability as defined by Chan *et al.* (2001):

Fraction I (very labile, VLC): Organic C oxidizable under $6.0 \text{ mol L}^{-1} H_2SO_4$

Fraction II (labile, LC): Difference in OC oxidizable under 9.0 mol L^{-1} and that under 6.0 mol L^{-1} of H_2SO_4

Fraction III (less labile, LLC): Difference in OC oxidizable under 12.0 mol L^{-1} and that under 9.0 mol L^{-1} of H_2SO_4 ($12.0 \text{ mol L}^{-1} H_2SO_4$ is equivalent to the standard Walkley and Black method)

Fraction IV (non-labile, NLC): Residual organic C after oxidation with 12.0 mol L⁻¹ of H₂SO₄ when compared with TOC.

3.10.2.9.3 Total Carbohydrate carbon and its' water soluble fractions

Total carbohydrate carbon in soil samples were determined by anthrone method as described by Brink *et al.* (1960). For total acid hydrolysable carbohydrate carbon estimation, 5g of air dry soil was passed through 1 mm sieve and hydrolyzed for 24 hours on a steam bath (85°C) with 50 ml of 3N sulphuric acid. Hot hydrolysate was passed through fritted disc filter of medium pore size and residue was washed with 50 ml of hot water. The filtrate was cooled at room temperature and brought to 100 ml with distilled water. 5 ml of dilute soil hydrolysate was mixed with 10 ml of anthrone reagent and absorbance of the green colour was read at 625 nm against blank. Standard curve was prepared using standard glucose solution. Amount of carbohydrate carbon was obtained from glucose concentration of the sample multiplying with a factor of 0.4 (glucose contains 40% carbon).

For extraction of water soluble soil carbohydrate fractions the procedure given by Ghani *et al.* (2003) was adapted with slight modifications. 3g oven dry soil samples (three replications for each OTC) passed through 1 mm sieve were weighed into 50 ml polypropylene centrifuge tubes. 30 ml of distilled water was added and were shaken for 30 min on an end-over-end shaker at 30 rpm and at 20 °C. The tubes were then capped and left for 16 h in a hot-water bath at 80°C. At the end of the extraction period, each tube was shaken for 10s on a vortex shaker to ensure that hot water soluble carbohydrate fractions get released from the SOM were fully suspended in the extraction medium. These tubes were then centrifuged for 20 min at 3500 rpm. The supernatants were filtered through 0.45 mm cellulose nitrate membrane filters to analyse the total carbohydrate content of the soil extract by anthrone method.

3.10.2.9.4 Microbial Biomass Carbon (MBC)

Soil microbial biomass C (MBC) was estimated by fumigation extraction method as described by Vance *et al.* (1987). Two sets of freshly removed soil (approximately 17.5 g on dry weight basis) was taken in schott bottle and fumigated with ethanol-free chloroform for 24 hours and the excess chloroform was removed from one set by

repeated back suction, while other set was kept unfumigated. After incubation the bottle was placed in a fume hood at 40°C–50°C until chloroform evaporated completely. After removal of even traces of chloroform, 70 ml of 0.5 M K₂SO₄ was added to fumigated and unfumigated samples. The bottles were then shaken in an end-to-end shaker for 30 minutes. The extract was filtered through Whatman No. 42 filter paper. optical density (OD) of the fumigated and unfumigated extract at 280 nm was taken immediately and the microbial biomass carbon was measured as per the method (Nunan *et al.*, 1998), using aliquots of K₂SO₄ extracts through dichromate digestion. Microbial biomass carbon was calculated after back titration with ferrous ammonium sulphate and calculated using the equation:

$$\text{Biomass C } (\mu\text{g/gm of soil}) = 2.64 \times \text{CE}$$

where, CE = (organic C from fumigated soil) – (organic C from unfumigated soil). Microbial biomass C was expressed as $\mu\text{g C g}^{-1}$ soil.

3.10.2.9.5 Mass loss-on ignition (LOI)

For measurement of mass loss-on ignition (LOI), 200 mg of soil was added to Silica crucibles, placed in a muffle furnace at 550°C for 4h, cooled to room temperature in desiccators, and weighed. LOI was measured as the difference between the oven-dry soil mass and the soil mass after combustion, divided by the oven-dry soil mass (Schulte and Hopkins, 1996) and soil carbon content was determined from residual ash% by following the formula assuming the dry weight of % organic C on an average is 56% of organic matter (Hesse, 1971).

$$\% \text{ Carbon} = (100 - \text{ash } \%) / 1.74$$

3.10.3 Soil carbon dioxide flux

Carbon dioxide was sampled and analysed using Infra-red based continuous soil CO₂ flux analyser (LI-8100). LI-8100 system was used with a 20 cm short term survey chamber to obtain soil CO₂ flux. The closed chamber was placed on the soil and the rate of increase of the chamber CO₂ concentration was used to determine the soil flux. CO₂ diffuses out of the soil in response to the concentration gradient between the soil

pore spaces and the atmosphere. As the chamber CO₂ concentration raises, the concentration gradient between the soil and the chamber air decreases. This causes the measured soil CO₂ flux to decrease exponentially with time. The desired value of the soil flux was determined. The flux was estimated using the initial slope of a fitted exponential curve at the ambient CO₂ concentration. This is done to minimize the impact of the altered CO₂ concentration gradient across the soil surface after the chamber was closed.

3.11 Nodule Activity Assessment

At 45 days (Mann *et al.*, 2002) after sowing (DAS), considered the most active phase of nitrogen fixation for chickpea nodules, uniformly growing plants were randomly uprooted in five replicates from each OTC with minimum root injury and washed for free of soil. After physical assessment uniformly developed nodules were removed and kept on ice until use for further processing. For nodulation study, separate chickpea plants were grown in separate polythene lining (2m×1m) within the OTC plant population, other than core sampling for root parameter study. The total root system was separated for determination of nitrogenase activity and after that the nodules were detached afresh from chickpea root system and noting their fresh weight by precise digital balance. Nodules were scanned under win-RHIZO scanning system for determination of all physical characters immediately. After that, part of the active matured nodule were separated, weighed separately and masticated repeatedly in 80% ethyl alcohol for 15 minutes and centrifuged at 10000 rpm for 5 minutes. The supernatant was stored in 4°C collected for immediate biochemical analysis of leghaemoglobin, proline and phenol content. Some portions of nodules collected separately were dried at 65°C and then powdered for carbohydrate content and elemental analysis of C and N. All of the above experimentation was done in 4 replicates from each OTC for both the years of experimentation

The nitrogenase activity was determined by the acetylene reduction assay, adopting the procedure of Hardy *et al.* (1968). Nodulated roots were cut and shaken slowly to remove the adhering soil particles. Samples were assayed in 30-ml glass tubes sealed with a subseal which was pierced by hypodermic needle, and 10% (v/v) of the air was withdrawn from the sample container and replaced by an equal volume of acetylene gas. After 1 hour of incubation at room temperature, 0.5 ml of gas sample

was injected into a gas chromatograph (Nucon Series 5250, New Delhi, India), equipped with a flame ionization detector to quantify the ethylene produced. The result was expressed in terms of nanomoles of ethylene formed/g (nodule fresh mass)/hour.

Total nitrogen content of powdered nodule samples was determined by the micro Kjeldhal method as described above. The soluble protein content, in leaves and in seeds, was determined by the method of Lowry *et al.* (1951). The samples were homogenized in DDW and 5% trichloroacetic acid was added to precipitate the proteins. The precipitate was dissolved in 1% NaOH solution. Blue colour developed by using Folin phenol reagent and the absorbance was read at 660 nm, using spectrophotometer.

The proline content in fresh nodule samples was determined by following the method of Bates *et al.* (1973). Samples were extracted in sulphosalicylic acid. In the extract, an equal quantity of glacial acetic acid and ninhydrin were added. The sample was heated at 100 °C and toluene (5 cm³) was added to the sample. The absorbance by toluene layer was read at 528 nm on UV-VIS spectrophotometer.

The leghaemoglobin content in fresh nodules was quantified as described by Appleby and Bergersen (1980). It was extracted with sodium phosphate buffer (pH 7.4). The extract was divided equally into two test tubes, each one added with same volume of alkaline pyridine reagent. The hemochrome formed, was read at 539 and 556 nm on a spectrophotometer after adding a few crystals of potassium hexacyanoferrate and sodium dithionite, respectively.

Water-soluble carbohydrate (WSC) was determined by the anthrone method (van Herwaarden *et al.*, 1998), where carbohydrates were extracted from 50 mg of dried powdered nodule with 4 ml of 80% (v / v) ethanol at 80°C followed by two extractions each with 4 ml of water at 60°C. After centrifugation at 4000 rpm, for 20 min, 1 ml aliquot was taken out in six replicate from each and 4 ml of anthrone reagent was added with eight minutes heating in a boiling water bath. Then cooled rapidly and intensity of dark green color was read at 630 nm using UV-VIS spectrophotometer.

For total carbohydrate analysis of nodules, 100 mg of the ground nodule sample was weighed in six replicate into boiling tubes. The hydrolyzation was done by keeping the tubes in boiling water bath for 3 hours with 5 ml of 2.5 N HCl and

cooled to room temperature and was neutralized with solid sodium carbonate until the effervescence ceased. The volume was adjusted to 100 ml and 1 ml aliquot was taken out for total carbohydrate analysis by the anthrone method described above in section (3.7.5), after centrifugation at 4000 rpm for 10 minutes (Hedge and Hofreiter, 1962).

Nodule starch content was analyzed according to Sadasivam and Manickam (2008). 100 mg of the powdered nodule sample from each treatment in six replicates for each was homogenized in hot 80% ethanol to remove sugars, centrifuged at 4000 rpm for 10 min and the residue was retained. The residue was washed repeatedly with hot 80% ethanol till the washing do not give color with anthrone reagent and the residue was dried well over a water bath. To the residue 5.0 ml of water and 6.5 ml of 52% perchloric acid was added for extraction at 0°C for 20 min and centrifuged. The extraction was repeated using fresh perchloric acid and 0.2 ml of the supernatant was pipette out and the volume was made up to 1 ml with water. 4 ml of anthrone reagent was added to each tube and heated for 8 minutes in a boiling water bath. The intensity of green to dark green color was read at 630 nm using UV-VIS spectrophotometer.

Total phenol content in the nodules was determined with the Folin-Ciocalteu reagent (Malick and Singh, 1980). Phenols react with phosphomolybdic acid in Folin-Ciocalteu reagent in alkaline medium and produce blue colored complex (molybdenum blue). 0.5 g of the sample was weighed in five replicates for each OTC and was ground with a pestle and mortar in 10-time volume of 80% ethanol. The homogenate was centrifuged at 10,000 rpm for 20 min and supernatant was collected. Re-extraction was done for the residue with five times the volume of 80% ethanol, centrifuged and pooled the supernatants. The supernatant was evaporated to dryness and the residue was dissolved in 5 ml of distilled water. 0.5 ml of aliquots was taken out into test tubes and volume was adjusted to 3 ml with water. 0.5 ml of Folin-Ciocalteu reagent was added. After 3 min, 2 ml of 20% Na_2CO_3 solution was added to each tube and mixed thoroughly. The tubes were placed in boiling water for exactly 1 minute, cooled and the absorbance at 650 nm was measured against a reagent blank. A standard curve was prepared using different concentrations of catechol and from the standard curve the concentration of phenols was found out in the test samples and expressed as mg phenols/100g material in terms of catechol used as standard.

3.12 Statistical Analysis

The measurement data of soil parameters and nodule activity were statistically analyzed using the SPSS statistical software Version 16.0 (SPSS Inc., Chicago) and the significance of differences between mean values was analyzed statistically by analysis of variance (ANOVA) procedure as given by Zar (2010).

4. RESEARCH PAPER 1

Growth behaviour of *kabuli* chickpea under elevated atmospheric CO₂

Abstract

An open top chamber (OTC) study was conducted on *kabuli* chickpea (cv. 1105) for two crop seasons in 2010-11 and 2011-12 to study the impact of elevated atmospheric carbon dioxide (580±20 ppm) on its growth performance, leaf pigmentation and biomass partitioning. Chickpea responded positively to the CO₂ elevation in terms of increased plant height, higher Leaf Area Index (LAI) and leaf chlorophyll content. The leaf carotenoids content along with specific leaf area was found to decrease under elevated CO₂ with more dry matter partitioned towards the stem portion. The result suggests more vigorous growth of plant canopy with thicker greenish and long lived chickpea leaves, under future elevated CO₂ atmosphere.

Keywords: open top chamber, chlorophyll, biomass, leaf area index

4.1 Introduction

The carbon dioxide (CO₂) concentration in the atmosphere has increased by at least 37.27%, since the start of the industrial revolution (Keeling *et al.*, 2008). Authoritative forecasts claim that the average carbon dioxide (CO₂) concentration in the terrestrial troposphere will continue to increase up to 570 µmol mol⁻¹ by the middle of the current century with a predicted consequence of 2.0 to 4.5°C warmer earth surface (IPCC, 2007). Since CO₂ is an important causal factor of global climate change, and also the substrate for photosynthesis, any change in the atmospheric CO₂ concentrations impacts plant growth by enhancing the carboxylation process and reducing oxygenation; thereby changing its partitioning efficiency i.e. carbon allocation in various plant organs (Liu *et al.*, 2005).

In their pot experiment of Canberra phytotron, Morison and Gifford, (1984) found that atmospheric CO₂ enrichment of 560 ppm increased leaf area with reduced transpiration rate for 16 different agricultural and horticultural species. Greater leaf area along with higher total plant dry mass at all growth stages of wheat was reported under 600 ppm level of atmospheric CO₂ enrichment (Pal *et al.*, 2005). Elevated CO₂ was reported to increase the leaf size, plant height and fresh and dry mass of shoots in berseem (Pal *et al.*, 2004). Qiao *et al.* (2010) reported more tillers in winter wheat

(*Triticum aestivum* L. cv. Kenong 9204) grown in OTC for 712 ppm level of elevated atmospheric CO₂.

Variable response was reported for LAI under the influence of elevated CO₂. In their open top chamber study, significant increase in LAI was reported for wheat (Tausz-Posch *et al.*, 2012) and pigeon pea (Saha *et al.*, 2012) due to atmospheric CO₂ enrichment. In contrast, reducing trend of LAI in atmospheric CO₂ enrichment was reported for maize (Kim *et al.*, 2007). The leaf chlorophyll content of wheat was found to be increased (Demothes and Knoppik, 1994), unaffected (Donnelly *et al.*, 2000) or decreased (Cheng *et al.*, 2010) due elevated CO₂ exposure.

Chickpea (*Cicer arietinum* L.) is the largest produced food legume in South Asia and the third largest produced high-quality food legume globally. South and Southeast Asia region including India, Pakistan and Iran, shares three-fourths (~81%) of the world chickpea area and production (~77%). India is the largest chickpea growing country in the world. India accounts Asia's 73.3% share of production and 72% of area for chickpea, with an average productivity of 801 kg ha⁻¹ (Rao *et al.*, 2010).

A large number of publications are available focusing on the influence of atmospheric CO₂ enrichment on the growth behavior of several cereals, but very few for the pulses. Being an important C₃ legume of semi-arid region, information on the influence of elevated CO₂ concentration on growth behavior of chickpea in Indian context is rarely reported. Hence, the present study was planned to investigate and quantify the impact of elevated CO₂ on chickpea.

4.2 Materials and method

4.2.1 Site characteristics

The experimental site is situated in semi arid climate of New Delhi, at 28°35'N latitude, 77°12'E longitude and at 228.16 m above mean sea level with a fairly leveled topography. The average maximum and minimum air temperatures during crop growth season (Rabi 2010-11 and 2011-12) were 19.5°C and 6.6°C, respectively. The average annual rainfall of the study area was about 710 mm, of which 80% is received during southwest monsoon from July to September. The mean daily rainfall was 3.3 mm/day during the crop growth period. The site has sandy loam soil with 69.98 % sand and 21.2 % clay.

4.2.2 Experimental details

The circular structures of OTCs, fabricated using aluminium frame as used by Pal et al. (2004), were installed in the field. The diameter and height of each OTC were 3.0 and 2.5 m respectively. The frame was lined with polyvinyl chloride (PVC) sheets which transmitted 85% of natural sunlight. Upper part of OTCs had a frustum of 0.5 m at 2.5 m height and the base of each OTC was provided with a hollow ring of 0.4 m diameter covered with PVC sheet and perforation inside for supply of CO₂ and air mixture inside the OTCs. CO₂ supply and monitoring: Pure CO₂ gas (99.7%, v/v CO₂) procured from M/S Gas Associates, New Delhi, was released from a commercial grade cylinder fitted with a regulator (DURA, make ESAB, India) through solenoid valve and PV tubing connected to an air-exhaust blower mounted at the base of each OTC. To maintain a desired level of CO₂ in two OTCs at 580±20 µmol mol⁻¹ at crop canopy height, continuous injection of pure CO₂ into plenum of OTCs was done where it was mixed with air from air compressor before entering into the chamber. The air sample from the middle of the chamber was drawn periodically into a CO₂ sensor (NDIR, make Topak, USA) and the desired level of CO₂ was maintained with the help of solenoid valves, Program Logic Control (PLC), and Supervisory Control and Data Acquisition (SCADA) Winlog software (Make SELCO Italy). The CO₂ data logging, control and operation were performed by a PC through DOIP (digital input and output module) on real time basis. In two of the OTCs, elevated levels of CO₂ were maintained from 6 AM to 6 PM since the emergence of the crop. Two OTCs were used as chamber-controls without any external supply of CO₂, but with similar circulation of air through the blower.

4.2.3 Crop Agronomy

Chickpea seeds (*Cicer arittinum* L.; cv. Pusa 1105- *kabuli* type) were treated with the fungicide captan @ 2 g kg⁻¹ of seed, with *Rhizobium* culture and 10% sucrose solution in shade. The seed were sown manually in rows with a spacing of 30 cm row-to-row and 15 cm plant-to-plant, respectively. Basal dose of 20-40-60 NPK was applied during seed bed preparation and the sowing operation was completed within third week of November. After 3 weeks, plants were thinned a density of 20 plant/m² in each OTC.

4.2.4 Destructive sampling and detailed experimentation

To study the dry matter partitioning and subsequent crop growth analysis, the above ground plant parts were harvested randomly in triplicate from each OTC at three distinct crop growth stages. viz. initial vegetative, 50% flowering and pod maturity stage . Based on physical appearance, the specific crop growth stages appeared at 50, 94 and 125 DAS for 2010-11 and 48, 72 and 90 DAS for 2011-12 respectively. The harvested plants were washed and the leaves were separated after recording of the morphological characters (plant height, fresh biomass production). The same plant samples were used for measurement of biomass and the leaf area index. The green leaf portions of the cut plants were separated and the total leaf area per plant was measured using leaf area meter (LICOR-100) and LAI was calculated as:

$$\text{LAI} = \frac{\text{Measured leaf area per plant (cm}^2\text{)} \times \text{Number of plants per m}^2}{100 \times 100 \text{ (cm}^2\text{)}} \quad \dots (4.1)$$

Leaf chlorophyll (Chl) content was determined by using a non-maceration technique of Hiscox and Israelstam (1979) in which 1 g leaf slices were kept overnight in 10 ml of dimethyl sulphoxide (DMSO), the absorbance were recorded at 645 and 663 nm in spectrophotometer and the chlorophyll content was calculated using the equations of Arnon (1949). For leaf carotenoids content absorbance was taken at 480 nm wavelength and derived from the equations proposed by Kirk and Allen (1965). For measurement of mass loss-on ignition (LOI), 200 mg of soil were added to Silica crucibles, placed in a muffle furnace at 550°C for 4h, cooled to room temperature in desiccators, and weighed. LOI was measured as the difference between the oven-dry soil mass and the soil mass after combustion, divided by the oven-dry soil mass (Schulte and Hopkins, 1996) and soil carbon content was determined from residual ash% by following the formulae assuming the dry weight of % organic C is on an average 56% of organic matter (Hesse, 1971). For calculating dry matter yield four plants were randomly selected in each OTC and cut at ground level. Different plant parts like leaf, stem, and pod were separated. Those plant materials were oven-dried at 65°C for 48 hours and weighed separately by precise digital balance. Dry matter in different plant parts was expressed in g m⁻² unit. Root and stem portions are then collected for both elevated and ambient CO₂

exposed plants and ignited at 450°C for 6 hrs in a muffle furnace to estimate the mass loss in ignition (%).

Ash content (%) as fed basis = (weight of residual ash × 100) / weight of sample

Ash content (%) adjusting to dry basis = (Ash content (%) as fed basis × 100) / dry matter % as on fed basis

Where Dry matter % = (weight of dry sample at 105°C × 100) / weight of samples before drying

Mass loss on ignition (%) = (1 - % ash content) ... (4.2)

Carbon % = (100 - ash %) / 1.8 ... (4.3)

4.2.5 Statistical analysis

The data were analyzed statistically using completely randomized design and the significance was tested at 5% and 1% level of critical difference using SPSS version 16.0.

4.3 Results and Discussion

The plant height was 1.2 to 1.5 times (Fig. 4.1) higher under elevated CO₂ with a prominent increase in LAI (Fig. 4.2). The effect was much more prominent at 50% flowering and active pod filling stage. Elevated CO₂ accelerated the plant growth by 44.64% after 94 DAS during 2010-11 and 12.92% after 72 DAS during 2011-12, with a significant increase in LAI at 0.05 and 0.01 levels of significance. Initial vegetative stage was found to be less sensitive to the atmospheric CO₂ fertilization. The growth enhancement effect of chickpea may be enhanced nutrient uptake including nitrogen by chickpea plants with increased photosynthetic assimilation process (Bhatt *et al.*, 2007). Increased canopy photosynthesis enables plants to utilize more photosynthates for its growth with concomitant increase in chickpea LAI, resulting from enhanced N uptake. As chickpea is leguminous crop with nitrogen fixing nodules (Singh and Diwakar, 1995). There is possibility of increased nitrogen fixation under elevated CO₂ that was utilized by chickpea plants to support the process of growth enhancement (Gamper *et al.*, 2005).

In spite of increase in total leaf area by 1.45 times in 2010-11 and 2.08 times in 2011-12 along with the leaf weight by 1.62 and 3.63 time in 2010-11 and 2011-12

respectively, the Specific Leaf Area (SLA) was found to be decreased by 19.33% in 2010-11 and 22.45% in 2011-12 at 50% flowering stage (Fig. 4.3a). Our result supports the finding of 159% increase in wheat leaf area at 90 day under elevated CO₂ (Pal *et al.*, 2005) and 43.72% increase in plant height with a 3.34 fold increase in LAI of *Cenchrus ciliaris* fodder species (Bhatt *et al.*, 2007). In contrast to the findings of Bhatt *et al.* (2007), our result strengthen the finding of decreased wheat SLA (Pal *et al.*, 2005) under elevated CO₂, indicating the less expansion of the chickpea leaf area per unit dry matter accumulation. Although amplified magnitude of chickpea LAI was supported by the increase in total leaf area per plant for CO₂ fertilization treatments along with individual leaf weight, but the magnitude of weight increase overshadows the area expansion magnitude in both of the years. This observation also supports the findings of increased leaf thickness due to higher rate of photosynthesis under elevated CO₂ (Das, 2003). Our result thus indicates the dominance of the process of more photosynthetic carbon assimilation due to enhanced photosynthesis over individual leaf nitrogen assimilation for chickpea under elevated CO₂ environment.

During the entire crop growth stages stem dry weight was found to be 1.5 to 2.8 times higher under elevated CO₂ treatment and at 50% flowering stage, it was found to be increased under elevated CO₂ by 47.9 and 141.8% during 2010-11 and 2011-12 respectively (Fig. 4.3b). Mass loss in ignition data (Fig. 4.4a) at 50% flowering stage showed a significant increase in carbon allocation towards chickpea roots by 5.3% ($p < 0.01$) during 2010-11 and 3.6% ($p < 0.01$) during 2011-12, Whereas non-significant increasing trend for stem allocation was recorded under elevated CO₂ condition, which may signifies the enhanced downward movement of photosynthates resulting from increased photosynthetic assimilation under elevated CO₂ environment. On the other hand, increased mass loss in ignition (Fig. 4.4b) by 8% and 19% ($p < 0.05$) for ambient CO₂ treatments was signified due to the presence of more carbonaceous materials in the soils exposed to ambient CO₂ during two consecutive years of our experimentation. (Saha *et al.*, 2011). Thus, significant increase in chickpea growth and more dry matter allocation towards the stem under elevated CO₂ was due to the photosynthetic enhancement effect of CO₂ enrichment and the partitioning of photosynthates towards different growing aboveground plant organs (Pal *et al.*, 2004), The extra carbon fixed by the plants due to CO₂ enrichment

was found to be translocated to growing axis of the plant species (Sharma and Sengupta, 1990).

Significant increase in leaf chlorophyll content (Fig. 4.5a) along with decreasing trend of leaf carotenoids content (Fig. 4.5b) was found under elevated atmospheric CO₂ enrichment over the entire crop growth season in both of the years. The accumulation of leaf Chlorophyll was increased by 18.94 to 32.72% ($p < 0.01$) under elevated CO₂ during 2010-11, where as for the second year it was ranged between 8 to 12% ($p < 0.05$) increases only at different crop growth stages. Leaf carotenoids content showed almost reverse trend with 3 to 6 % ($p < 0.05$) decrease in 2010-11 along with 14 to 30% ($p < 0.01$) decrease during 2011-12. Our result was further supported by the elevated CO₂ induced protection against leaf chlorophyll damage, ensuring the greenness of the leaves for a longer duration (Donnelly *et al.*, 2000). It may be an indirect indicator of more nitrogen utilization under elevated CO₂ along with decreasing trend of leaf carotenoids content was a prominent indicator of increasing self-life of leaf chlorophyll in chickpea with enhanced photosynthesis under elevated CO₂ environment. Wang *et al.* (2004) reported that elevated CO₂ increased chloroplast number per unit cell area. Being a legume, chickpea can fulfill its N requirement through symbiotic nitrogen fixation and thus our result can be explained as with no limitation of supply in plant available N, plant N uptake was found to be accelerated under elevated CO₂ with an increase in the foliar N concentration and leaf chlorophyll content (Cheng *et al.*, 2010).

4.4 Conclusions

Our result supports a significant positive growth response of chickpea to the elevated atmospheric CO₂ enrichment. Decreasing trend in SLA suggest the occurrence of less expanded thick green leaves for a longer duration under elevated CO₂ condition. As chickpea N₂ fixing legume so increased biomass allocation and other growth attributes for above ground parts impose the possible beneficial effect of elevated CO₂ for chickpea due to possible increase in individual plant height, LAI, leaf chlorophyll content and dry matter yield, decrease in leaf carotenoids content.

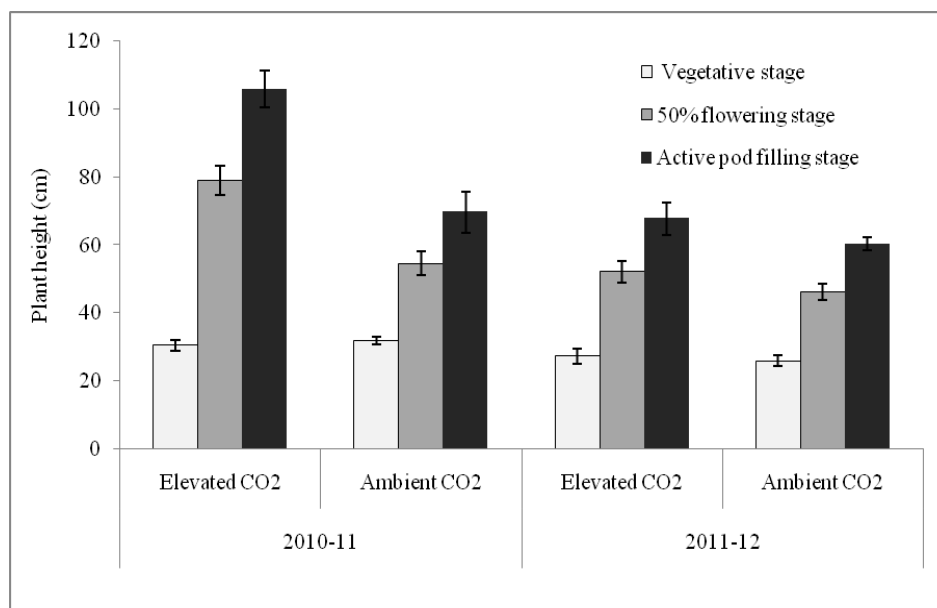


Fig. 4.1: Variation of plant height during different crop growth stages in chickpea

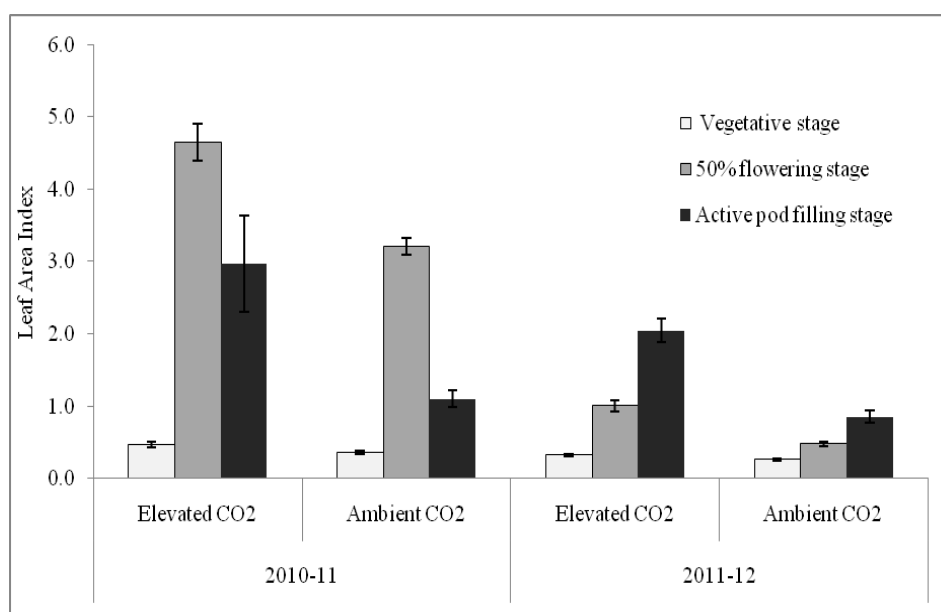


Fig. 4.2: Variation of Leaf Area Index (LAI) during different crop growth stages in chickpea

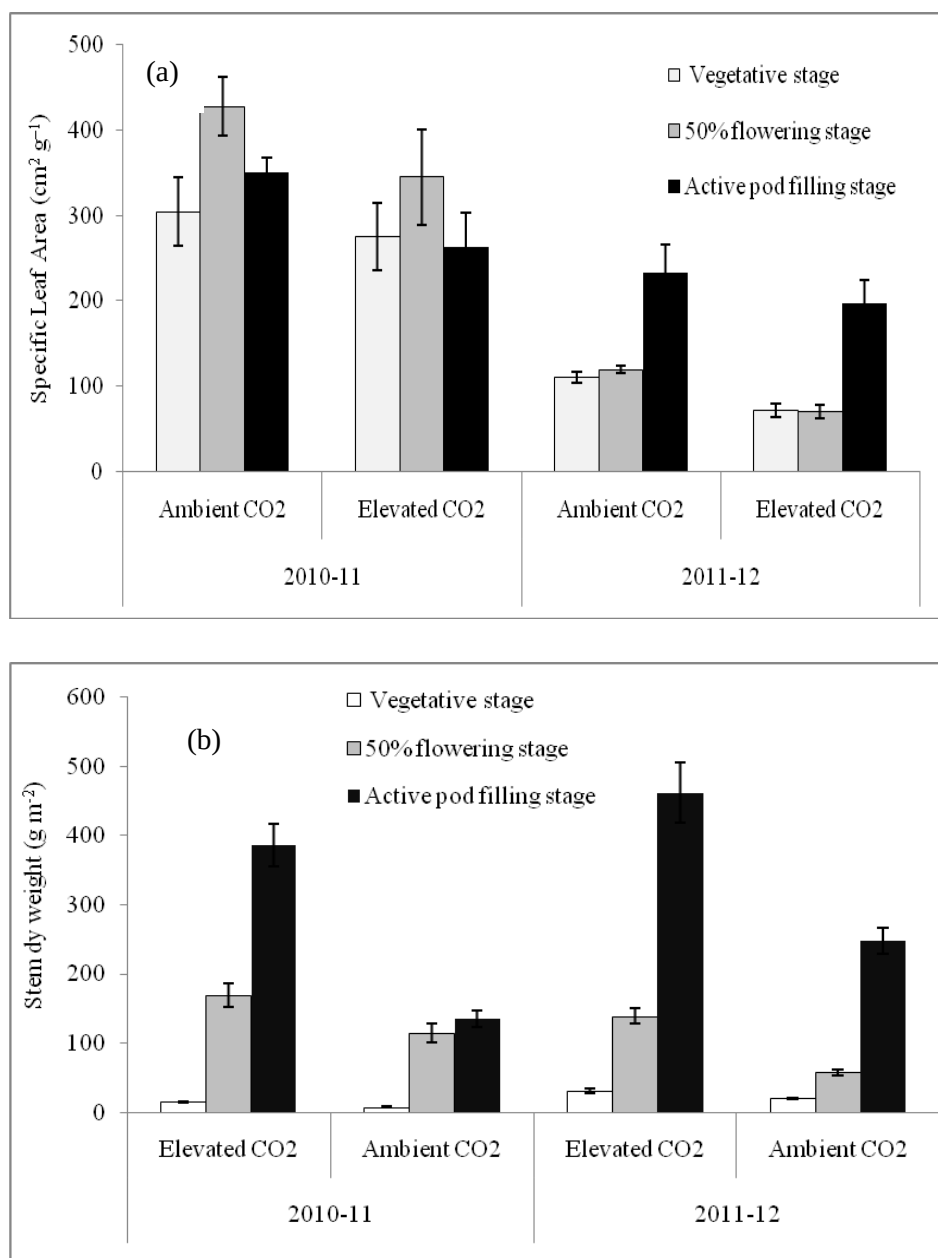


Fig. 4.3: Variation of (a) SLA ($\text{m}^2 \text{g}^{-1}$) and (b) stem dry weight (g m^{-2}) during different crop growth stages in chickpea

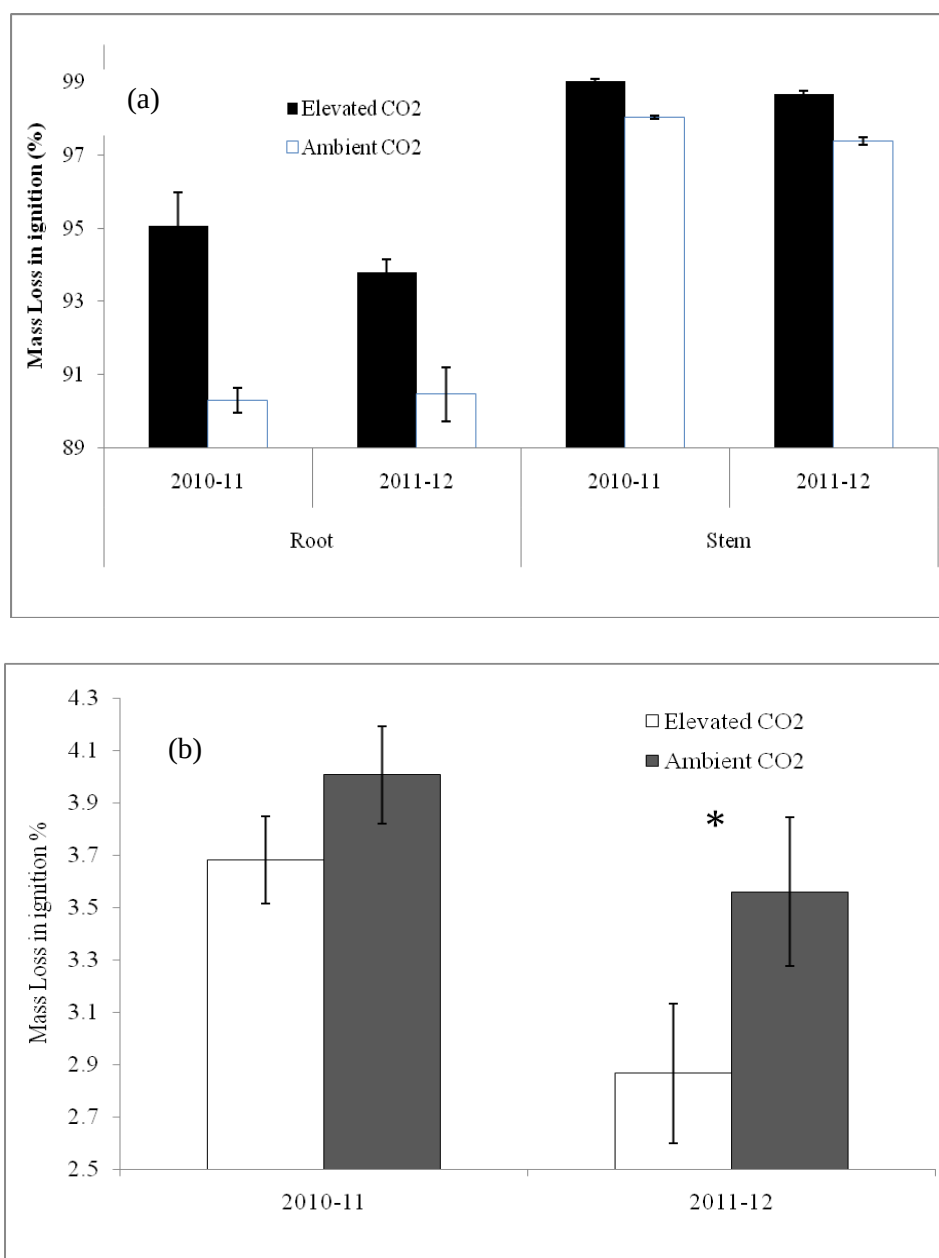


Fig. 4.4: Variation of mass loss in ignition for (a) root and stem portions of chickpea at 50% flowering and (b) soil exposed to elevated and ambient CO₂ treatments.

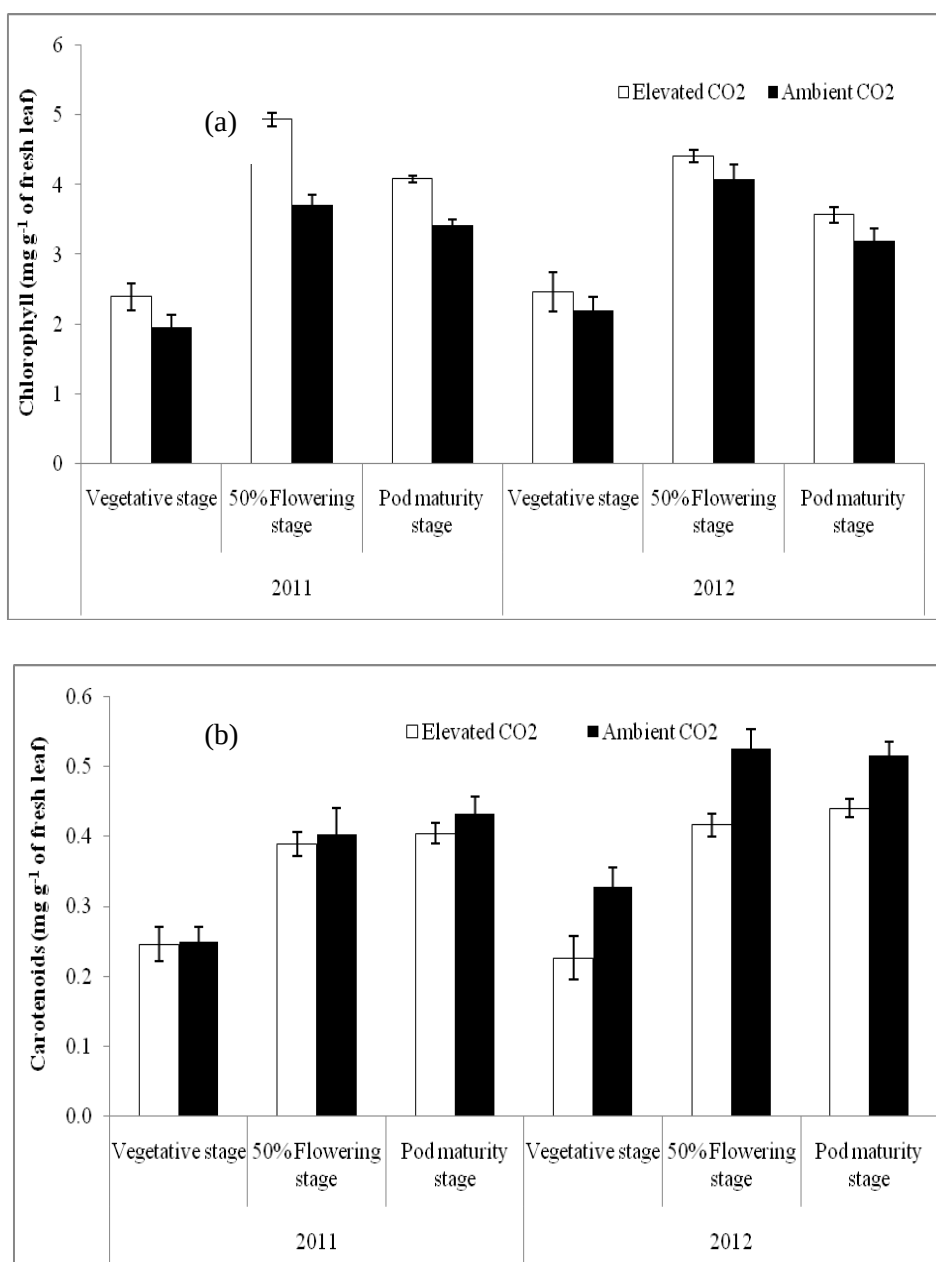


Fig. 4.5: Variation of leaf pigmentation (a) total chlorophyll (mg of Chl / gm of leaf fresh wt) and (b) total carotenoids mg of carotenoids/g of leaf fresh wt) during different crop growth stages in chickpea.

5. RESEARCH PAPER 2

Atmospheric carbon dioxide enrichment induced modifications in canopy radiation utilization, growth and yield of chickpea [*Cicer arietinum* (L.)]

Abstract

Response of chickpea (Pusa-1105 genotype) was evaluated through an open top chamber experiment in Indian Agricultural Research Institute, New Delhi, to study the differential response towards the atmospheric CO₂ enrichment at 580±20 ppm level with subsequent changes in plant canopy structure and other associated plant biophysical characteristics, for two consecutive year 2010-11 and 2011-12. The magnitude of leaf area index (LAI) amplification was reported about 22.6% for elevated CO₂ treatment at post flowering period. But no significant change in fractional intercepted photosynthetically active radiation (IPAR) was recorded along with a significant reduction in the value of canopy extinction coefficient (*k*) by 18.5% under elevated CO₂. The radiation use efficiency (RUE) was found to be 135.3% higher over ambient CO₂ exposed plant due to higher biomass production as well as higher grain yield along with maintenance of higher Specific Leaf Nitrogen (SLN) values, although the Specific Leaf Area (SLA) got reduced under elevated CO₂. Increased area per unit leaf under elevated CO₂ indicates the possibility of occurrence of thicker leaf lamina due to elevated CO₂ exposure. Increased level of water soluble carbohydrate (WSC) in leaf bears the obviousness of more carbon assimilation due to higher photosynthetic activity under elevated CO₂ with widening of leaf C:N ratio at 50% flowering stage. Elevated CO₂ facilitates more allocation of leaf N and soluble carbohydrate fraction towards the economic yield fraction and ultimately that was resulted into a significant increase in Harvest Index (HI) for chickpea crop due early pod set and increased pod yield per unit plant but no change in individual seed dimension. Thus it is essential to modify the radiation-based crop growth models with changed utilization coefficient of PAR for elevated CO₂ exposed chickpea plants by taking account the variability in IPAR under elevated atmospheric CO₂ enrichment.

Key words: Carbon-dioxide, open-top chamber, radiation use efficiency, extinction coefficient, specific leaf nitrogen, harvest index

5.1 Introduction

In our today's industrialized world, the major attention is taken out by general public and the scientific community, focusing on the detrimental effect of increasing atmospheric green house gases (GHGs) on the terrestrial ecosystem, threatening the sustainability of future crop production. Atmospheric concentrations of carbon dioxide have shown a steady exponential increase with an average annual growth rate of 1.4 ppm per year *in situ* values at Mauna Loa, from 315.98 ppm in 1959 (Keeling *et al.*, 2009) to a current global mean of 391.38 ± 0.13 ppm to our atmosphere (Le Quéré, 2012). It is forecasted to reach at the level of $570 \mu\text{mol mol}^{-1}$ in atmosphere by the middle of this century (IPCC, 2007). The increase in atmospheric CO_2 concentration has both positive and negative impacts on crop growth and development either directly or indirectly. The projected increase of atmospheric CO_2 concentration is expected to enhance carbon assimilation rates (Wu *et al.*, 2004; Finnan *et al.*, 2005) affecting the growth, development and yield of important agricultural crops (Cure and Acock, 1986), by modifying the biochemical and ecophysiological functioning of leaves and structural elements of the plant canopy that control solar radiation absorption (Dermody *et al.*, 2008; Drewry *et al.*, 2010a). At present, there is an urgent need to quantitatively assess impacts of atmospheric CO_2 enrichment on different field crops.

Elevated CO_2 was found to have a consistent fertilization effect on plant aboveground biomass as well as seed yield, thus modifying the Harvest Index (HI), especially for C_3 plants (Kimball *et al.*, 2002; Long *et al.*, 2004). Maximum Leaf Area Index (LAI) was reported to increase due to CO_2 fertilization in open top chamber (OTC) experiments for sorghum (Ottman *et al.*, 2001), soybean (Dermody *et al.*, 2008; Sun *et al.*, 2009), rice (Zeng *et al.*, 2011), wheat (Tausz-Posch *et al.*, 2012) and pigeon pea (Saha *et al.*, 2012). In contrast, reducing trend of LAI under atmospheric CO_2 enrichment was reported for maize (Kim *et al.*, 2007), sugarbeet (Manderscheid *et al.*, 2009) and *Echinochloa crusgalli* L. (Zeng *et al.*, 2011). Short term exposure of elevated CO_2 resulted in a significant increase in biomass production for rice without change in LAI (Anten *et al.*, 2003). Sims *et al.* (1999) also found no significant difference in total canopy leaf area under elevated CO_2 level of $700 \mu\text{mol mol}^{-1}$ for sunflower. Short term exposure of elevated CO_2 was reported to a significant increase in biomass production for rice without change in LAI (Baker *et al.*, 1992; Imai *et al.*,

1985; Anten *et al.*, 2003). Ewert (2004) reviewed the relatively smaller interactions between LAI and elevated CO₂ effects on canopy assimilation. Lower N availability was found to be the effective cause of suppressing the response of LAI towards elevated CO₂ (Arnone and Körner, 1995; Franklin and Ågren, 2002). In contrast, Weigel and Manderscheid (2012) reported about no statistically significant CO₂ effect on LAI for barley and wheat but significant reduction for ryegrass. Elevated CO₂ was also found to modify the temporal pattern of plant LAI response by delaying the LAI loss to senescence in soybean (Dermody *et al.*, 2006) and early attainment of peak LAI in pigeon pea (Saha *et al.*, 2012).

Reports examining the impact of elevated atmospheric CO₂ on canopy radiation interception and its use efficiency in well-watered peanut stands under elevated CO₂ level of 700 $\mu\text{mol mol}^{-1}$ (Clifford *et al.*, 1993), offsetting the negative effects of water deficit. Higher CO₂ assimilation was found to be positively correlated with RUE in peanut (Bennett *et al.*, 1993), which enabled the crop plants to accumulate more dry matter more efficiently in expense of intercepted canopy radiation. Finally that was resulted a net increase in RUE for several cultivated crop species like Sunflower (Hui *et al.*, 2001), wheat (Monje and Bugbee, 1998; Manderscheid *et al.*, 2003), corn (Rudorff *et al.*, 1996), winter barley (Manderscheid *et al.*, 2009). Manderscheid *et al.*, (1997) showed that the elevated CO₂ effect on dry matter production of clover does not depend on temperature but on the intercepted radiation only. They found a curvilinear increase in RUE from 2.7 g MJ⁻¹ to 3.9 g MJ⁻¹ between 380 and 660 $\mu\text{mol mol}^{-1}$ CO₂ due to the positive CO₂ effect on the quantum use efficiency of photosynthesis. Smart *et al.* (1994) observed an increase in LAI with reduction in extinction coefficient (*k*) under elevated CO₂. Sims *et al.* (1999) indicated that the CO₂ induced increase in LAI may be nullified with subsequent reduction in the extinction coefficient. Manderscheid *et al.* (2003) in their open top chamber study found no effect of elevated CO₂ treatment on seasonal light absorption in wheat when grown with sufficient water and nutrient supply. De costa *et al.* (2006) found a sharp decrease in rice RUE, under elevated CO₂ during the post-heading period, and explained it as an effect of reduction in leaf nitrogen concentration. Weerakoon *et al.* (2000) reported an increase in RUE of *Oryza sativa* L. (cv. IR72) along with increased light interception under elevated CO₂ during early crop growth stages in open top chamber. Manderscheid *et al.* (2009) reported the

ameliorative effect of CO₂ enrichment on decreased seasonal radiation absorption by the green canopy under limited nitrogen supply. In an open top chamber grown pigeon pea (*Cajanus cajan* L.), 52.3% increase in RUE was reported along with change in canopy extinction coefficient with increased crop LAI, under elevated CO₂ in open top chamber condition (Saha *et al.*, 2012).

Supporting the findings of Monje and Bugbee (1998), Pal *et al.* (2003) reported increased partitioning of carbon towards leaf with reduction in leaf N that resulting in increase in carbon to nitrogen ratios (C:N) under elevated CO₂ for soybean. High CO₂ induced leaf N reduction was found to disappear with enhanced N supply and lowering the C:N ratio (Roger *et al.*, 1998). Higher total N uptake for elevated CO₂ exposed crop plants are also reported (Srivastava *et al.*, 2001). Aranjuelo *et al.* (2011) showed an increase in flag leaf C:N ratio with decreases in leaf N in response to elevated CO₂. Jain *et al.* (2007) found the increase in leaf carbon content with a concomitant decrease in leaf N concentration for Spinach and fenugreek grown under elevated CO₂. Elevated CO₂ was reported to decrease tissue nitrogen content causing wider C:N ratios (Stiling and Cornelissen, 2007). In contrast, Newingham *et al.* (2013) reported about no increase in foliar C:N ratios due to elevated CO₂ exposure. Low nitrogen availability was found to be constrains the response of growth response of *Betula pendula* under elevated CO₂, (Esmeijer *et al.*, 2009). Cheng *et al.* (2010) recorded the promotion of photosynthetic energy transport rate and assimilatory ability with higher N availability under elevated CO₂ concentration. Ainsworth *et al.* (2004) identified the genetic capacity of a plant species to be a crucial factor for the ability of plants to sustain increased photosynthesis and utilization of photosynthates when grown under elevated CO₂.

Atmospheric CO₂ enrichment was also reported to accelerate the senescence of barley flag leaves due to increased grain nutrient sink capacity and accelerating leaf N release to the growing seed (Fangmeier *et al.*, 2000). Crop biomass production and seed yield is the product of photosynthetic solar energy conversion. In contrast elevated CO₂ had no significant effect on biomass production and seed filling (Aranjuelo *et al.*, 2011) elevated CO₂ was found to be the major stimulating factor for significant increase in aboveground plant biomass for berseem (Pal *et al.*, 2004), wheat (Högy *et al.*, 2010; Tausz-Posch *et al.*, 2012), barley (Wall *et al.*, 2011), *Brassica napus* L. (Frenck *et al.*, 2011; Franzaring *et al.*, 2011), rice (Zeng *et al.*,

2011), pigeon pea (Saha *et al.*, 2011), soybean (Lam *et al.*, 2012b), due to the increased partitioning of photosynthetic assimilated carbon towards the growing organs of plants. Regarding economic yield, elevated CO₂ was found to increase wheat seed yield by 13% due to increased tiller numbers and seed mass. The number of seeds per head was decreased resulted from a sustained increase in canopy quantum yield (Monje and Bugbee, 1998). Wu *et al.* (2004) found an increase in yield under elevated CO₂ treatment, mainly due to an increase in the number of wheat grains per plant. Li *et al.* (2000) reasoned that the increase in wheat grain weight under 550 $\mu\text{mol mol}^{-1}$ level of elevated CO₂ was due to a faster rate of grain filling. Besides, cultivar specificity of the enhanced seed yield factor was observed for soybean (Ziska *et al.*, 2001), spring wheat (Manderscheid and Weigel, 1997) and *Brassica napus* L. (Frenck *et al.*, 2011).

Imai *et al.* (1985) found an increase in rice harvest index with 700 $\mu\text{mol mol}^{-1}$ CO₂ due to increase of ripening period by about six days. In contrast, atmospheric CO₂ enrichment levels of 580 and 680 $\mu\text{mol mol}^{-1}$, were found to cause a decline in rice harvest index (Ziska *et al.*, 1997; Kim *et al.*, 2001). De costa *et al.* (2006) found about no effect of elevated CO₂ on rice harvest index, in spite of greater percentage of filled grains under elevated CO₂. Increasing trend of harvest index, resulting from more partitioning of photosynthetic assimilates to grains was reported for wheat grown under 684 $\mu\text{mol mol}^{-1}$ level of CO₂ enrichment (Wheeler *et al.*, 1996). In contrast, slightly declining trend was found by Manderscheid *et al.* (2003, 2009) for wheat harvest index under 660 and 550 $\mu\text{mol mol}^{-1}$ elevated CO₂ level and no influence of elevated CO₂ levels of 689 and 1200 $\mu\text{mol mol}^{-1}$ on wheat harvest index (Manderscheid and Weigel, 1997; Monje and Bugbee, 1998; Mulholland *et al.*, 1998). Cure *et al.* (1986) reviewed that the variation in harvest index was small and erratic in most crops except for soybean, where it decreased with doubling of CO₂ concentration. Sun *et al.* (2009) supported this finding by averaging 8 years experimental data in SoyFACE, of which showed a with 3% decrease in soybean harvest index along with 18% and 13% increase in biomass and seed yield, respectively. Increased harvest index of broad bean (*Vicia faba*) was reported under elevated CO₂ level of 700 $\mu\text{mol mol}^{-1}$ (Vanaja *et al.*, 2008). In contrast, harvest index of pigeon pea was found to be decreased irrespective of cultivar specificity reasoned as the lack of plant's capacity to utilize completely the more abundant photosynthetic

assimilates under elevated CO₂ condition (Saha *et al.*, 2012). Aranjuelo *et al.* (2013) explained the increase in wheat yields was not been due to an increase in total biomass but rather due to an increase in harvest index.

It is evident that crop RUE should increase due to increase in total crop biomass under elevated CO₂ condition. But there is lack of information lacking and many contradictions, while describing the actual reason behind that increase. There are several contrasting possibilities contributing for increase in RUE under elevated CO₂ condition. For atmospheric CO₂ enrichment, either increased LAI may increase the canopy radiation interception, by lowering the magnitude of increase in RUE value under elevated CO₂ or modification in leaf orientation and canopy architecture. This may compensate the effect of increased LAI, resulting no change or decrease in canopy radiation interception. Thus it will modify the crop RUE value. Otherwise there is a possibility that no change or even decrease in LAI due to elevated CO₂ induced nitrogen deficiency and it will offset the beneficial effect of elevated CO₂ on crop RUE. Our work was aimed to evaluate the best possibilities among these three.

Available literature has reported the effect of elevated CO₂ on radiation utilization pattern of several crops, mostly for cereals, oilseeds and closed forest canopy. But for field grown pulse, it is scarcely reported. Chickpea [*Cicer arietinum* L.] is an important winter-season pulse and a cheapest source of protein for millions of families in developing countries (Joshi *et al.*, 2002). It is the third most important food legume having a share of 14.6% in global pulse production (FAOSTAT, 2010), grown on 15.3% of the total global pulses area, adding 8.8 million tons to the global food basket., India is the largest producer of chickpea, accounting for 64% of the global chickpea production (Gaur *et al.*, 2010). Up to our knowledge domain, some field-scale research literatures are available investigating the radiation interception characteristics for chickpea, but none under atmospheric CO₂ enrichment condition. Therefore, it is relevant to examine the possible changes in radiation interception and utilization pattern of chickpea in relation to its growth behavior, under CO₂ enriched environment in subtropical India. In the light of above, a study was conducted to evaluate the carbon capture and its conversion efficiency by chickpea under atmospheric CO₂ enrichment.

5.2 Material Methods

5.2.1 Site description

The experiment was conducted in open-top chambers (OTCs) on a fairly leveled topography on chickpea (*Cicer arietinum* L.; cv. Pusa 1105- *kabuli* type), indeterminate growth habit during *rabi* season (mid November- mid April) of 2010-11 and 2011-12, at Indian Agricultural Research Institute, New Delhi, located at 28°35' N latitude, 77°12' E longitude and at an altitude of 228.16 m above mean sea level (MSL).

5.2.2 Climate

The climate of the site was characterized by a typical semi-arid with dry hot summer and mild winters with an average annual rainfall of 710 mm, of which 80% is received during southwest monsoon from July to September. The mean daily maximum and minimum air temperature was between 10-29°C and 2-12°C respectively. The mean daily evaporation (USWB open pan evaporation) was 1.8 mm/day during the crop growth period.

5.2.3 Soil

The surface soil (0-30 cm) of the experimental site was non-calcareous and sandy loam in texture with 68.8 % sand 19.2% silt and 12.2 % clay. The average bulk density of the soil was 1.61 Mg m⁻³; Water holding capacity 32.6%; saturated hydraulic conductivity, 1.17 cm h⁻¹; pH (1:2.5 soil: water suspension) 7.9; electrical conductivity, 0.46 dS m⁻¹; organic C, 0.03 g kg⁻¹; total N 0.031%; and available P and K, 5.046 and 453.86 kg ha⁻¹, respectively.

5.2.4 Experimental details

In two OTCs, elevated levels of 580±20 µmol mol⁻¹ CO₂ were maintained from 6:00 AM to 6:00 PM from the crop emergence and the other two OTCs were used as chamber controls without any external supply of CO₂. The circular structure of OTCs was fixed in the field and fabricated using aluminum frame as described by Saha *et al.* (2011). Pure CO₂ gas (99.7%, v/v CO₂ and less than 10 µmol mol⁻¹ CO) was released from a commercial grade cylinder fitted with a regulator (DURA, make ESAB, India)

through solenoid valve and PV tubing connected to air-exhaust blower mounted at the base of each OTC. During the entire period of experiment temperature and humidity inside all the four chambers were continuously recorded with automatic sensors (Model TRH 511, Ambetronics, Switzerland) fitted in the middle of each OTC. That was connected to data logger (Model TC 800D, Ambetronics, Switzerland).

5.2.4.1 Crop Management practices

Chickpea seeds were treated with the fungicide captan @ 2 g kg⁻¹ of seed. In the next day, seeds were surface sterilized by dipping in 0.1 % HgCl₂ for 2 min. After rinsing in sterile distilled water, seeds were allowed to imbibe water for 6h and were then coated with *Rhizobium* culture, and 10% sucrose solution in shade. The seed were then sown manually at 10 cm depth on conserved soil moisture by putting 2–3 seeds per hole in rows and maintaining 30 cm the row-to-row spacing and 15 cm plant-to-plant spacing. Basal dose of 20-40-60 NPK was incorporated during seed bed preparation. The sowing operation was performed in 1st week of November, 2010 and 1st week of December 2011. After 3 weeks of sowing operation, the newly emerged plants were thinned and allowed to grow with a density of 20 plant m⁻² in each OTC. Weeding was done manually, while fungicide mixture (Bavistin + Dithane M-45) was sprayed at 50 days after sowing (DAS) to prevent the fungal infection. The crop was grown as a rainfed crop, but two irrigations, one each at branching and pod filling stages was applied. The date of floral bud initiation and the date of physiological maturity were recorded based on visual appearance for the OTC grown chickpea plants.

5.2.4.2 Weather during crop growth

Daily maximum and minimum temperatures, mean RH, total radiation and other meteorological parameters were recorded in the nearby meteorological observatory of the Indian Agricultural Research Institute, New Delhi. Mean daily maximum and minimum temperatures and relative humidity (RH %) inside the four OTCs was recorded using data logger.

5.2.4.3 Leaf area index (LAI)

Leaf area index (LAI) was measured at weekly intervals with a plant canopy analyzer (LAI-2000, LI-COR, Lincoln, NE, USA). The measurement of LAI was performed around 4:30 pm in six replicates taken from evenly spaced spots in two diagonal transects in each OTC to maintain almost fixed incident solar angle, with higher proportion of diffuse incident light at sunset.

5.2.4.4 Canopy radiation extinction coefficient (k)

The daily estimation for amount of incoming PAR (MJ m^{-2}) was calculated from global solar radiation (obtained from pyranometer), as proposed by Caviglia *et al.* (2004):

$$\text{PAR} = 0.48 * \text{global solar radiation (MJ m}^{-2}\text{)} \dots\dots\dots(4.1)$$

Intercepted Photosynthetically Active Radiation (IPAR) by the whole canopy was measured weekly by using apogee point quantum sensor SQ-215 model on clear sunny days between 11:30 - 12:30 hours IST. Eight measurements were averaged for each chamber, four with inter-row placement and the other four with intra-row placement. The fractional interception of PAR (fIPAR) was calculated as

$$\text{fIPAR} = (I_0 - I) / I_0 \dots\dots\dots(4.2)$$

Where, I_0 = Incident PAR on the top of the canopy, I = Transmitted PAR at the bottom of the canopy.

Radiation interception through crop canopies follows an exponential-type attenuation process as (Varlet-Grancher *et al.*, 1982; Rosenberg *et al.*, 1983) as given below,

$$\text{fIPAR} = 1 - e^{(-k \times \text{LAI})} \dots\dots\dots(4.3)$$

Canopy radiation extinction coefficient (k) were calculated with least-square regression by calculating the slope of the relationship between $\ln(1 - \text{fIPAR})$ and LAI (Saha *et al.*, 2012).

5.2.4.5 Radiation Use Efficiency (RUE)

The daily incident solar radiation, recorded by pyranometer, was multiplied with a factor of 0.48 to calculate daily incident PAR. The fIPAR at each day after sowing was multiplied with daily incident PAR to calculate intercepted PAR. The intercepted PAR was then summed over a specific crop growth period to calculate the cumulative intercepted radiation, which was further corrected for 85% transmittance of sunlight through the polythene cover of OTC. Radiation use efficiency (RUE) was calculated as the slope of the plotted linear relationship between total biomass accumulation for a specific crop period and cumulative intercepted radiation during that period and expressed in the units of g MJ^{-1} unit.

5.2.4.6 Plant sampling

In order to study the dry matter yield and to determine the crop RUE, the above ground plant parts were harvested randomly in four replicate from each OTC at three distinct crop growth stages, viz. vegetative, 50% flowering and active pod filling stage. The crop was considered to have reached a particular growth stage when 50% of the plants had reached that stage. The specific crop growth stages appeared at 50, 94 and 125 DAS for 2010-11 and 48, 72 and 90 DAS for 2011-12.

5.2.4.7 Leaf nitrogen dynamics study

Specific leaf area (SLA) was calculated by using the formula as suggested by Ma *et al.* (1992). To derive the specific leaf area (SLA; leaf area per leaf dry matter in $\text{m}^2 \text{g}^{-1}$), leaves were separated from other plant parts and the total leaf area per plant was measured using leaf area meter (LICOR-100). The leaves per plant were counted and then oven-dried at 65°C for 48 hours and weighed precisely by a digital balance. The dried leaf samples were then ground, to a fine powder (1 mm). A 100 mg sub sample was withdrawn and analysed for leaf nitrogen content using micro-Kjeldahl digestion. Specific leaf nitrogen (SLN) in gN m^{-2} per unit leaf area was calculated as the ratio of leaf nitrogen content and specific leaf area (Muchow, 1988) at different crop growth stages, along with leaf C:N ratio at 50% flowering stage only. The total carbon was analysed by vario TOC Analyzer (Version 09.07.2010) of Elementar Analysensysteme GmbH.

5.2.4.8 Water soluble carbohydrate analysis

To determine the water soluble leaf carbohydrate content, 100 mg dry leaf sub sample was taken out and shaken in 10 ml hot water for 30 min at 80°C in a water bath (Manderscheid *et al.*, 2009). After filtration, the carbohydrates concentration in the aqueous solution was determined by the anthrone method (Yemm and Willis, 1954).

5.2.4.9 Yield component analysis at harvest

In both of the seasons, flowering of chick pea was accelerated by about 4-5 days and the maturity came one week earlier for the elevated CO₂ exposed plant than one control. At the end of the crop growth season, final harvesting of the crop was performed to assess the seed yield at harvesting maturity, when the entire plant dried out and appeared a yellowish brown appearance. In the first season, the ambient CO₂ exposed crop plants attained their harvesting maturity in 144 days after sowing. But in the second season, the delayed sowing had shortened the length of the crop growth period by 30 days and harvesting maturity was attained in 114 days after sowing for the same. After harvest, leaves and stem portions were separated from the pods and oven dried to constant weight at 80°C for around 48 hours their dry weights were recorded by a precise digital balance. Pods were sundried and several plant parameters viz. pod number, seed number and number of seeds per pod was recorded on per plant basis.

5.2.4.10 Statistical analysis

The experiment was conducted in a completely randomized block design (CRD) with four replications for each ambient and elevated CO₂ treatment. Statistical analysis of the data was done by two sample t test for two-tailed hypothesis using SPSS statistical software Version 16.0 (SPSS Inc., Chicago). For comparison of two slopes, to evaluate the values of the canopy extinction coefficient and RUE, Zar-t test was adapted (Zar, 2010).

5.3 Results

5.3.1 Crop growth as affected by elevated CO₂

Atmospheric CO₂ enrichment modified the phenological response of chickpea crop. Flower initiation advanced under elevated CO₂ by 4 days in the first crop growing season and 7 days in next year. In continuation, elevated CO₂ exposed plants attained early pod maturity by 7 and 14 days for 2010-11 and 2011-12, respectively.

5.3.2 LAI response to elevated CO₂

During 2010-11, the canopy LAI was significantly higher (at $p < 0.05$) from 45 DAS till crop maturity for plants grown under elevated CO₂ than control (Fig. 5.1). As compared to ambient CO₂ treatment, peak LAI value was found to be 22.6% higher under elevated CO₂ at 111 DAS (post flowering stage) and thereafter it declined gradually as the crop matured. During the second season, the CO₂ induced amplification of crop LAI started to dominate 30 days after sowing. The magnitude of LAI amplification was reduced by almost 1.5 times as compared to previous year. In both the years, a rapid decline in LAI was observed during senescence phase for plants grown under elevated CO₂ than plants grown under ambient CO₂ (Fig. 5.1).

5.3.3 Radiation interception and its use efficiency

The fractional Intercepted PAR (fIPAR) increased with increase in LAI, initially at a higher rate and then at lower rate and ultimately flattening (Fig. 5.2a). In 2010-11, continuous increase in fIPAR was recorded up to 70 DAS and then it attained a plateau for both of the treatments, with no change in fIPAR afterwards. In 2011-12 also the fIPAR increased till 40 DAS and then attained a plateau. For both of the seasons, no significant change in cumulative canopy radiation interception was observed. For calculation of canopy extinction coefficient (k), $\ln(1-fIPAR)$ was linearly plotted against LAI at different growth stages. A linear relationship between the two was fitted by pooling the two year's data and the slope of the relationship was canopy extinction coefficient (k). The k was found to be significantly lower for the elevated CO₂ treatment (Fig. 5.2b). The values of k for Pusa-1105 chickpea variety were 0.745 and 0.914 for elevated and ambient CO₂ treatments, respectively. The

Zar-t test (Zar, 2010) showed that the two values of k are significantly different (at $p \leq 0.05$).

Similarly, two year's data of above ground biomass and cumulative intercepted PAR was pooled to derive the RUE. The slope of linear regression with zero intercept between above ground biomass and cumulative intercepted PAR gave the RUE (Fig. 5.3). The RUE value was 0.313 g MJ^{-1} for elevated CO_2 as compared to 0.133 g MJ^{-1} under ambient CO_2 with R^2 values of 0.80 ($p < 0.05$) and 0.88 ($p < 0.05$), respectively. The results indicate that elevated CO_2 treatment results in significant increase in chickpea RUE. The Zar-t value showed that the RUE value was 135.3% higher ($p \leq 0.05$) under elevated CO_2 than under control. The increase in above ground biomass under elevated CO_2 was the major factor contributing to increase in crop RUE.

5.3.4 Dynamics of Specific Leaf Nitrogen and Water Soluble Carbohydrate content

Figure 5.4 shows the average area per unit leaf at three crop growth stages for plants grown under elevated and ambient CO_2 treatments in two years. The results indicate that elevated CO_2 resulted in significant increase in area per unit leaf at vegetative and 50% flowering stages but reduced at pod maturity stage. It increased by about 14.3% and 11.4% ($p \leq 0.05$) time at vegetative stage in two years. The increase in area per unit leaf at 50% flowering was about 15.3% ($p \leq 0.01$) and 11% ($p \leq 0.01$) under elevated CO_2 in 2010-11 and 2011-12, respectively. The magnitude of decrease was at pod maturity stage was about 21.3% ($p \leq 0.01$) and 16.7% ($p < 0.01$) during two consecutive years of our experimentation.

Plants grown under elevated CO_2 showed a significant reduction in Specific Leaf Area (SLA) at three crop growth stages (Fig. 5.5). Average reduction in SLA over crop growth period was about 17.8% and 24.4% for the two successive years. Among the three growth stages, the highest decrease of 19.3% and 22.5% in SLA ($p \leq 0.01$) was found at 50% flowering stage. The decrease in SLA at vegetative stage in 2010-11 was % which is statistically non-significant but in 2011-12 the SLA decreased significantly by 34.8% ($p \leq 0.05$). The SLA also decreased significantly at pod maturity stage by 24.8% ($p \leq 0.01$) and 15.7% ($p \leq 0.05$) in both the years.

Specific Leaf Nitrogen (SLN) was computed as the ratio of leaf nitrogen content and specific leaf area (Muchow, 1988) in terms of mg of leaf N per unit leaf area at three growth stages and is shown in figure 5.6. The SLN increased at vegetative and 50% flowering stages but decreased at pod maturity for plants grown under elevated CO₂. The SLN increased by 16.1 % ($p \leq 0.05$) and 17.9% ($p \leq 0.05$) at vegetative stage and by 32.2% ($p \leq 0.01$) and 24.0% ($p \leq 0.01$) at 50% flowering stages in the two years. The magnitude of decrease in SLN at pod maturity was 31.6% ($p \leq 0.01$) and 37.5% ($p \leq 0.01$) for two years.

The total leaf nitrogen (on dry weight basis) showed no significant change under elevated CO₂ treatment at 50% flowering stage in the two years although total leaf carbon content increased by 7.4% and 15.4%. It resulted in a significant increase in leaf C:N ratio at 50% flowering stage (Fig. 5.7). During 2010-11, the increase in leaf C:N ratio was 1.16 times ($p \leq 0.01$) with a correspondence increase in grain C:N ratio by 1.27 times ($p < 0.01$) whereas during 2011-12 the increase was 1.32 time ($p \leq 0.01$) and 1.3 times ($p \leq 0.01$) for leaf and grain C:N ratio respectively, under elevated CO₂.

Elevated CO₂ exposed chickpea plants showed a significant increase in water soluble carbohydrate accumulation in leaves especially during vegetative and 50% flowering stages in both the years (Fig. 5.8). The increase was 1.2 times ($p \leq 0.05$) at vegetative crop growth stage in two years while the increase was 1.45 times ($p \leq 0.01$), elevated CO₂ exposed plants showed about 1.2 times increase (Fig. 4.8) in leaf carbohydrate accumulation for both of the years. At 50% flowering stage, it was higher by about 1.45 times ($p \leq 0.01$) during 2010-11 and by about 1.14 times during 2011-12. At pod maturity stage, the water soluble carbohydrate in leaves decreased under elevated CO₂ in both the years though the decrease was statistically significant only in 2011-12.

5.3.5 Yield component analysis at harvest

Figure 5.9 shows different yield components at harvest for ambient and elevated CO₂ treatments in two years. The vegetative dry matter per plant at harvest (i.e. stem and leaf weight) increased significantly ($p \leq 0.05$) under elevated CO₂ in both the years. Similarly, the total number of pods per plant, total pod weight per plant and seed

weight per plant also increased significantly ($p \leq 0.01$) under elevated CO₂. It resulted in significant increase in seed yield per plant by about 118% and 45% under elevated in CO₂ in 2010-11 and 2011-12, respectively. The seed yield (weight of seed per plant) increased significantly by 107.7% ($p \leq 0.01$) and 43% ($p \leq 0.01$) during two consecutive years of experiment, as the number of pods per plant increased significantly by 98% during 2010-11 and 53.2% during 2011-12. But, there was no significant effect of elevated CO₂ on number of seeds per pod and 1000 seed weight. Even though there was increase in vegetative dry matter, the harvest Index (HI) increased significantly by 26.5% ($p \leq 0.01$) and 13.4% ($p \leq 0.05$) under elevated CO₂ in 2010-11 and 2011-12, respectively.

5.4 Discussion

Open top chamber studies were conducted to study the effect of CO₂ enrichment on chickpea growth, radiation interception, use efficiency and yield. The elevated CO₂ level of $580 \pm 20 \mu\text{mol mol}^{-1}$ was chosen as IPCC (2007) has forecasted it to reach at the level of $570 \mu\text{mol mol}^{-1}$ in atmosphere by the middle of this century. Chickpea being an important winter pulse crop and with very few studies reported on the effect of increase in atmospheric CO₂, in this study we attempted detailed analysis of LAI, radiation interception, RUE, leaf carbon and nitrogen, yield and HI under elevated CO₂.

The LAI growth profiles observed in this study under ambient and elevated CO₂ are typical for any crop in which LAI increases during vegetative growth, then saturating at flower initiation and declining during senescence. During 2011-12, the LAI was lower throughout the growth period than during 2010-11 on account of late sowing by one about month (Haloi and Baldev, 1986). The increase in LAI of chickpea under elevated CO₂ is clearly a manifestation of CO₂ fertilization affect (Kim *et al.*, 2001), where plants produce more biomass due to higher availability of carbon and absence of any other resource's constraint. Similar findings have been reported by Daepf *et al.* (2001) for *Lolium perenne* and Oikawa *et al.* (2013) for soybean crop. The effect of elevated CO₂ was most dominant at peak LAI growth stage which increased by 22.6% ($p < 0.01$) in 2010-11 and 41% ($p < 0.01$) in 2011-12 over control. Our result was supported by the findings of elevated CO₂ induced

increase in maximum LAI in soybean (Dermody *et al.*, 2006; Dermody *et al.*, 2008) and pigeon pea (Saha *et al.*, 2012). A rapid decline in LAI during senescence under elevated CO₂ was observed (Fig. 5.1). De la Mata *et al.* (2013) have also reported early senescence in sunflower under elevated CO₂ due to CO₂ induced alteration of nitrogen metabolism boosting mobilization of nitrogen from leaves and thus triggering early senescence. Miller *et al.* (1997) suggested that photosynthesis acclimation in tobacco under elevated CO₂ due to source/sink imbalance resulted in early senescence. Chickpea being a legume crop, the reason for early senescence may be attributed to accumulation of photosynthates in leaves causing source/sink imbalance and thus early senescence.

No significant effect of elevated CO₂ on fIPAR was observed in chickpea (Fig. 5.2a) even though it significantly increased crop LAI (Fig. 5.1) in both the years of experimentation. As with fIPAR, the total cumulative IPAR over the growth period also did not change significantly under elevated CO₂. There are contradictory observations of effect of elevated CO₂ on fIPAR. Some studies have reported increase in fIPAR under elevated CO₂ in pigeon pea (Saha *et al.*, 2012), wheat (Mulholland *et al.*, 1998; Batts *et al.*, 1998) while other reported no change in fIPAR values due to elevated CO₂ in rice (De Costa *et al.*, 2006) and barley (Manderscheid *et al.*, 2009). The canopy extinction coefficient '*k*' decreased significantly under elevated CO₂ to 0.75 as against 0.91 for ambient CO₂. The observed values of *k* in this study were slightly lower than value of 1.02 reported by Tesfaye *et al.* (2006) for chickpea but were much higher than 0.4–0.61 reported by Hughes *et al.* (1987). A lower *k* indicates that chickpea leaf orientation becomes more erect under elevated CO₂ resulting in better penetration of PAR into the canopy (Brekke *et al.*, 2011). Similar results have been reported by Saha *et al.* (2012) for pigeon pea. The decrease in fIPAR due to lower *k* might have compensated the increase in fIPAR on account of CO₂ induced increase in LAI (Drewry *et al.*, 2010b).

Improvement in photosynthetic RUE is one of the promising alternatives to increase crop productivity in future and this study showed the potential beneficial effect of elevated CO₂ in enhancing chickpea RUE. Reduction in dry matter production with reduced radiation interception had been reported in chickpeas (Verghis *et al.*, 1999). The regressed value of RUE of 0.133 g MJ⁻¹ under ambient

CO₂ estimated in our study was much lower than any other RUE value reported in literatures for chickpea (0.30-0.93 g MJ⁻¹ by Hughes *et al.*, 1987; 1.4 g MJ⁻¹ by Leach and Beech, 1988; 1.0 g MJ⁻¹ by Soltani *et al.*, 2006; 0.25-0.87 by Jahansooz *et al.* 2007). As in most of the studies reported in literature for different crops, a significant increase in RUE (2.35 times) due to elevated CO₂ was observed in chickpea also. The increase in RUE may be due to higher biomass production per unit of radiation interception as seen by increase in LAI for most part of growth season. It may also be due to more vertical and longer branches and internodes that improves light distribution within the canopy with reduced PAR interception in the upper canopy (Sims *et al.*, 1999) while increasing PAR interception in the lower canopy resulting higher photosynthetic efficiency (Walcroft *et al.*, 2005) and thus enhanced RUE. Moreover, photosynthetic efficiency is higher due to increased total leaf surface in the canopy (Sadras, 1996; Brodrick, *et al.*, 2013).

The elevated CO₂ treatment caused more leaf expansion resulting in increase in area per unit leaf at all growth stages (Fig. 5.4) whereas it caused decrease in SLA (Fig. 5.5). Similar increase in leaf area per plant was also reported in rice (Ziska *et al.*, 1996) and *brassica* (Upreti and Mahalaxmi, 2000) and soybean (Ainsworth *et al.*, 2002) under elevated CO₂ condition, along with increase in leaf thickness with greater green tissue density (Rogers *et al.*, 1996; Pal *et al.*, 2005). Bhatt *et al.* (2007) has reported increase in SLA of fodder crop under elevated CO₂ but Pal *et al.* (2005) has reported decrease of SLA in wheat crop as was the case with chickpea in this study. Vu *et al.* (2001) also reported increase in specific leaf weight (i.e. decrease in SLA) in soybeans under elevated CO₂. The decrease in SLA under elevated CO₂ may be due to either decrease in leaf area or increase in leaf weight. In our case as area per leaf increased, the only possible reason of decrease in SLA is that weight per leaf increased in higher proportion than the increase in leaf area. It indicates that elevated CO₂ causes more storage of dry matter in leaves produced due to higher photosynthetic rate.

SLN is an important parameter controlling the leaf photosynthetic responses for different field-grown crops (Sadras, 1996; Sinclair *et al.*, 2000 and Milroy and Bange, 2003). It was found to be an important determinant of RUE of crops because of the dependence of photosynthesis on leaf N (Milroy *et al.*, 2001; Brodrick *et al.*,

2013) and is also linearly related to LAI (Sinclair and Shiralwa, 1993). An increase in SLN at vegetative and 50% flowering stages under elevated CO₂ was observed. The elevated CO₂ enhanced C assimilation might have caused increase N assimilation in leaves which was readily available from nodule N fixation to maintain leaf C:N ratio, as chickpea is a legume crop. So, the increase in RUE of chickpea under elevated CO₂ (Fig. 5.3) may in part may also be attributed to increase in SLN. It also implies that legumes are more efficient in converting elevated CO₂ into biomass than cereal and oilseed crops. Reduction in specific leaf nitrogen at pod maturity stage was found to be associated with seed filling (Hall *et al.*, 1995). A significant decrease in leaf N content ($p \leq 0.05$) and subsequent reduction in SLN values ($p \leq 0.01$) at pod maturity may be due to greater mobilization of leaf N towards growing pods under elevated CO₂ environment. This experimental finding also supports the findings by Gifford *et al.* (2000) and Pal *et al.* (2004).

It is the proportionate increase in leaf carbon content than increase in leaf N content that resulted in a significant increase ($p \leq 0.01$) in the C:N ratio of chickpea leaves at 50% flowering stage in both of the years of experimentation (Fig. 5.7). Similar effect on C:N ratio of chickpea seeds was also observed. Elevated CO₂ was also reported to have modifying effect on leaf characteristics and its carbon- nitrogen dynamics (Gifford *et al.*, 2000; Coley *et al.*, 2002). Under N inadequacy canopies were found to be more erectophile under elevated CO₂ (Brooks *et al.*, 2000). In CO₂ enriched environment, the plant available excess carbon in the environment, can alter the uptake and utilization of N in leaf (Kim *et al.*, 2006; Leakey *et al.*, 2009), resulting in change in N composition and partitioning of different plant parts and ultimately modifying the leaf C:N ratio (Thürig *et al.*, 2003; Rogers *et al.*, 2004; Ainsworth *et al.*, 2006). An imbalanced wider C:N ratio may accelerates the senescence by producing systemic signals to increase the nitrogen availability by releasing nitrogen from old leaves for the plants grown with elevated CO₂ (Zhu *et al.*, 2009; De la Mata *et al.*, 2012). The widening of C:N ration of chickpea implies that elevated CO₂ may cause deterioration of produce quality. Further, greater water soluble carbohydrates accumulation (Fig. 5.8) in leaf is indicative of photosynthetic rate acceleration (at vegetative and 50% flowering stage) under the elevated CO₂ (Pal *et al.*, 2004; Ainsworth and Long, 2005; Davey *et al.*, 2006). The reduction in water

soluble carbohydrates at pod maturity may be indicative for photosynthetic acclimation (Miller *et al.*, 1997).

The yield component analysis clearly showed that elevated CO₂ resulted in increase in vegetative and reproductive dry matter in chickpea. Some of parameters related to plant's sink capacity, such as number of seeds per pod and 100 seed weight, were not at all affected by elevated CO₂. But there was overall increase in plant's sink capacity in chickpea as shown by significant increase in numbers of pods per plant and thus number of seeds per plant. Our results are supported by the observed increase in yield attributes viz. seed yield, total number of pods, pod dry weight per plant reported for broad bean (Wu and Wang, 2000), rice (Kim *et al.*, 2003, Liu *et al.*, 2008, Yang *et al.*, 2009), wheat (Manderscheid and Weigel, 1997; Wu *et al.*, 2004; Högy *et al.*, 2009c), rapeseed (Franzaring *et al.*, 2008; Högy *et al.*, 2010b; Franzaring *et al.*, 2011) and barley (Manderscheid *et al.*, 2009). Similarly, increased seed yield with increased pod weight per plant and seed weight per branches were reported for soybean (Ziska *et al.*, 2001; Ainsworth *et al.*, 2002; Twine *et al.*, 2013) which are in conformity with our results for chickpea. In contrast, Ziska *et al.* (2007) for soybean and Vanaja *et al.* (2007) for blackgram have reported increased seed size under elevated CO₂ but no such effect was observed for chickpea.

The increase in seed yield by 107.7% and 43.0% in two years was on account higher dry matter production as well as significant increase in HI of chickpea in two years. The HI realized for chickpea grown under ambient CO₂ in this study were 0.35 and 0.31 in 2010-11 and 2011-12, respectively (Fig. 5.9). These HI values were found to be similar with the reported HI values of 0.31-0.43 (Leport *et al.*, 1999) and 0.31-0.36 (Tesfaye *et al.*, 2006), but slightly lower than values reported by others (0.39 -0.54 by Saxena *et al.*, 1987). The HI increased to 0.44 and 0.35 under elevated CO₂ in 2010-11 and 2011-12, respectively (Fig. 5.9). Contrary results of decrease HI have been reported for rice (Kim *et al.*, 2003), soybean (Cure *et al.*, 1986; Ziska *et al.*, 2001; Morgan *et al.*, 2005b; Sun *et al.*, 2009) and pigeon pea (Saha *et al.*, 2012) and no effect on lupin HI (Palta and Ludwig, 2000). Elevated CO₂ induced phenological modifications i.e. early flowering and early pod set, would increase the proportion of pod-set (Saxena *et al.*, 1987) that may have also caused higher chickpea HI value in this study.

Yield of a crop can be defined by the equation $Y = f(\text{IPAR}, \text{fIPAR}, \text{RUE}, \text{HI})$, where Y is the seed yield, IPAR is the total incident photosynthetically active radiation over the entire duration of the crop, fIPAR is the fraction of incident radiation intercepted by the crop, RUE is the radiation use efficiency and HI is the harvest Index. Modification in any of these factors may influence yield as well as crop productivity. The fraction 'fIPAR' depends on LAI and canopy architecture (Monteith, 1981). In our study, although no significant change in fIPAR was observed, but increase in crop RUE value by 135.3% along with increase in chickpea HI by 20% was found to increase the crop yield by 80.3% for Pusa-1105 *kabuli* chickpea cultivar, when grown under elevated CO_2 . Although seed yield is a function of several components, our results also confirmed that strong positive correlations between RUE and seed yield in grain legumes (Tesfaye *et al.*, 2006) is maintained even under elevated CO_2 .

5.5 Conclusions

As a legume, the nitrogen fixing capacity of chickpea may improve under elevated CO_2 condition, but it cannot ameliorate the elevated CO_2 induced increased C assimilation in different plant parts, that may resulted into more amount carbonaceous produce with reduced quality. Our study also reveals the beneficial effect of elevated CO_2 on chickpea. Mostly chickpea are grown under water scarce semi-arid condition during rainy season. Elevated CO_2 fertilization has the potential to increase its conversion efficiency of solar radiation into seed yield dominating over increase in harvested plant biomass. Thus there is a possibility for possible enhancement of radiation productivity in drylands for growing chickpea in future elevated CO_2 world due its increase harvesting index value under elevated CO_2 condition. Besides, the information obtained from this study will be valuable in fine-tuning the radiation-based crop growth models or routines under climate change scenarios by taking into account the effect of elevated CO_2 on chickpea radiation utilization, leaf C-N dynamics and HI. Increased RUE and HI under elevated CO_2 pauses a significant potential to increase our legume crop productivity (mostly chickpea) in the projected future elevated CO_2 world. But more carbonaceous produce indicates the possibility of its quality deterioration.

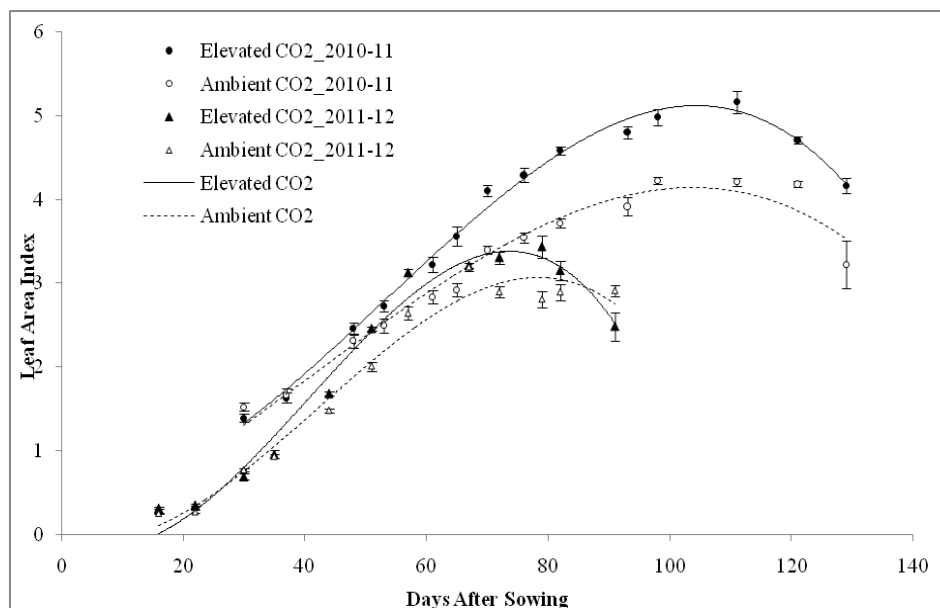


Fig. 5.1: Dynamics of chickpea LAI under ambient and elevated CO₂ conditions, for the entire crop growth period during 2010-11 and 2011-12.

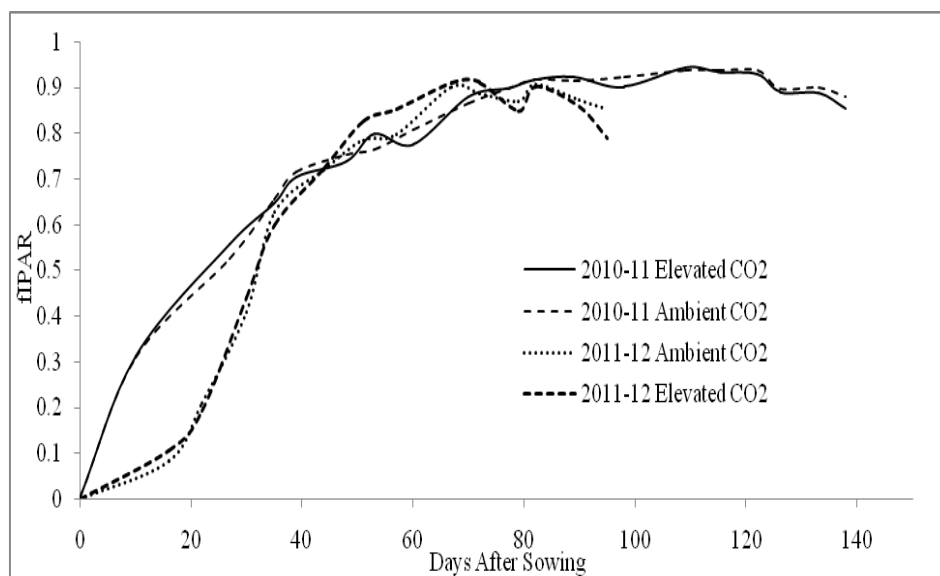


Fig. 5.2a: Variation in fIPAR during the entire crop growth period for chickpea under ambient and elevated CO₂ condition during 2010-11 and 2011-12.

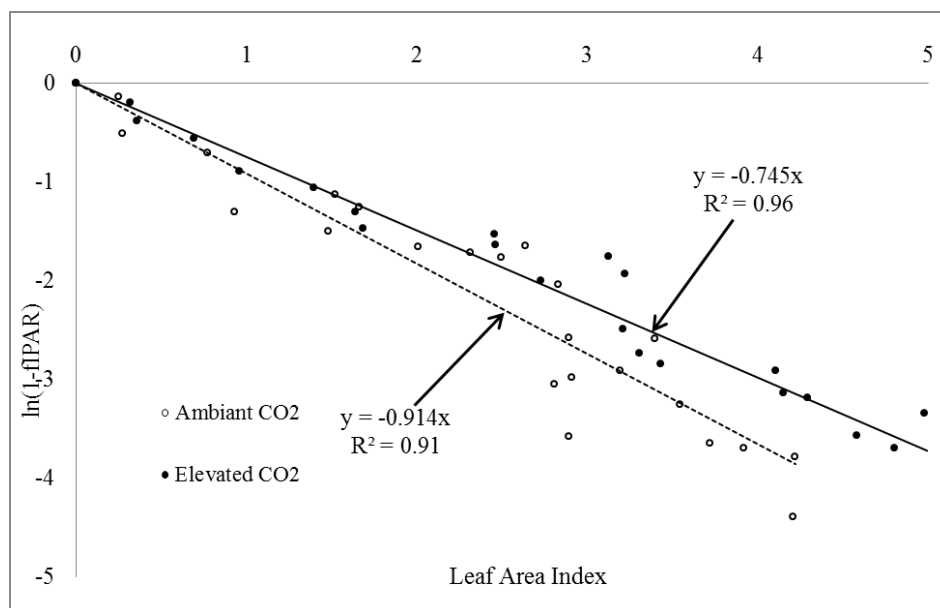


Fig. 5.2b: Linear regressive slope (k) between leaf area index and $\ln(1-fIPAR)$ for chickpea under ambient and elevated CO₂ condition.

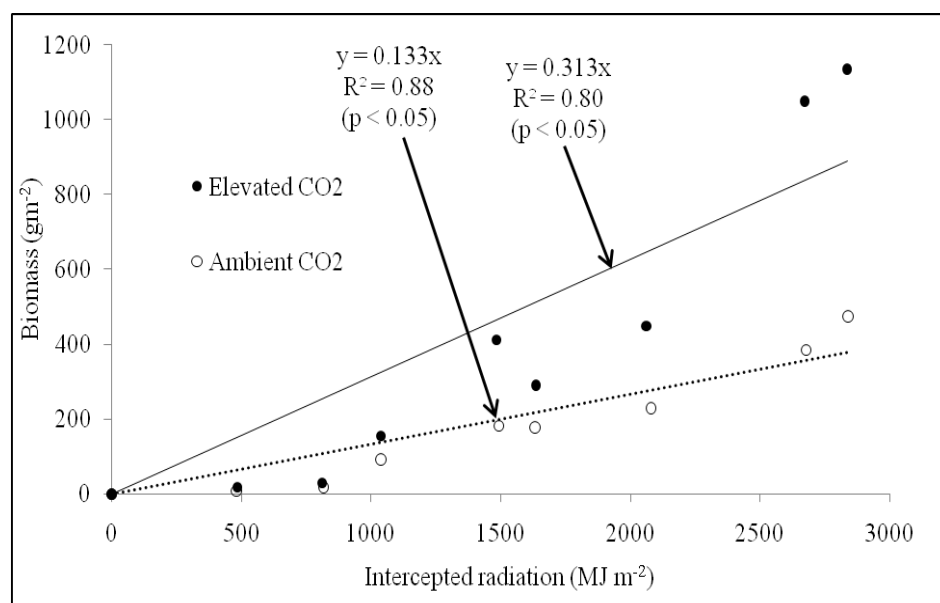


Fig. 5.3: Aboveground biomass of chickpea as a linear function of cumulative IPAR for different crop growth stages under ambient and elevated CO₂ condition

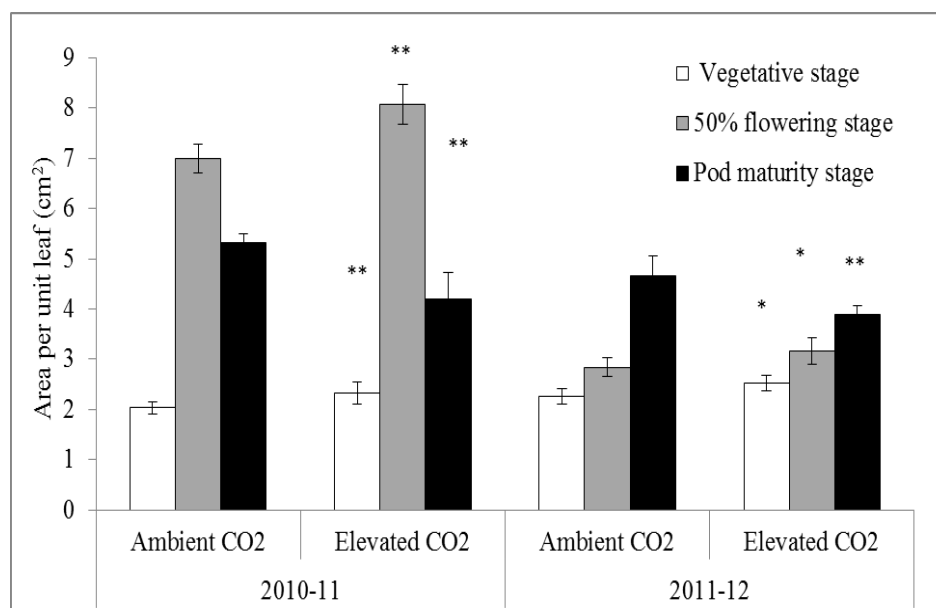


Fig. 5.4: Area per unit leaf of chickpea at different crop growth stages under ambient and elevated CO₂ condition during 2010-11 and 2011-12.

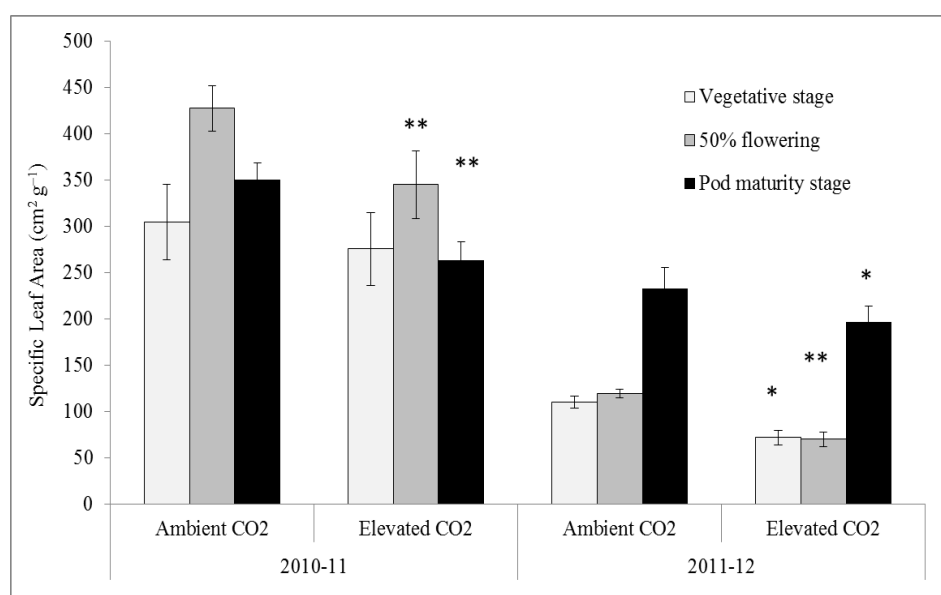


Fig. 5.5: Specific Leaf Area (SLA) of chickpea at different crop growth stages under ambient and elevated CO₂ condition during 2010-11 and 2011-12.

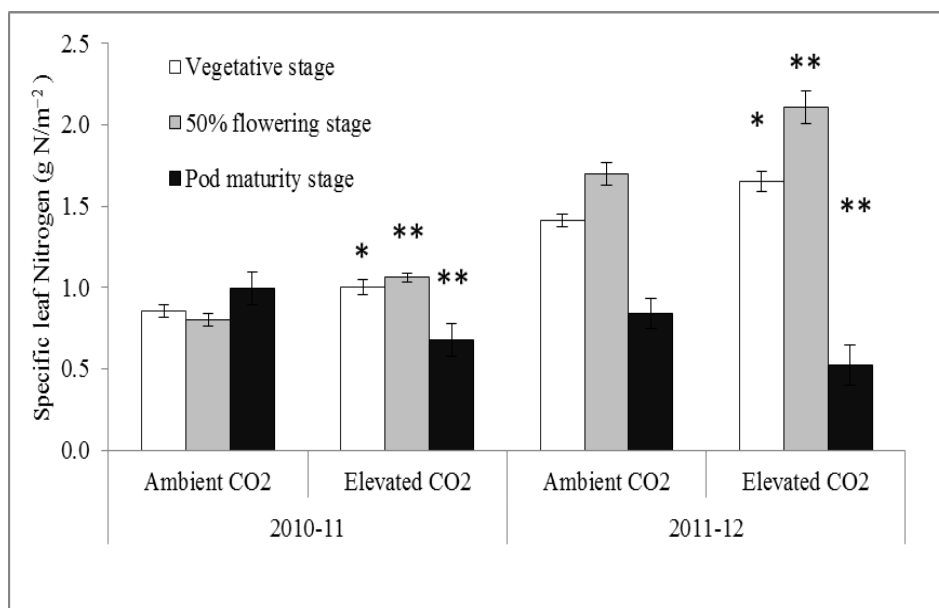


Fig. 5.6: Specific leaf N of chickpea at different crop growth stages under ambient and elevated CO₂ condition during 2010-11 and 2011-12.

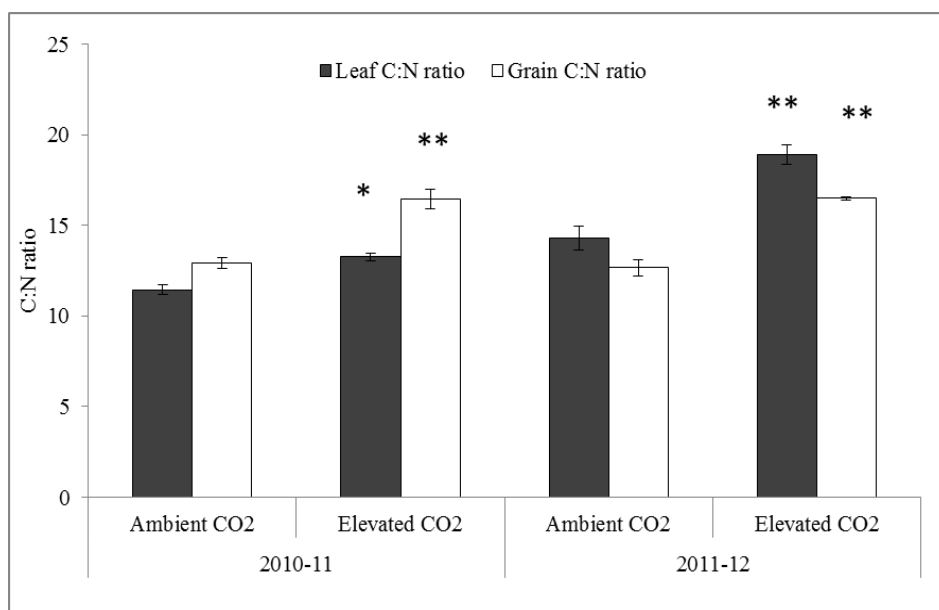


Fig. 5.7: Leaf C:N ratio (at 50% flowering stage) and grain C:N ratio (at harvest) of chickpea under ambient and elevated CO₂ condition during 2010-11 and 2011-12.

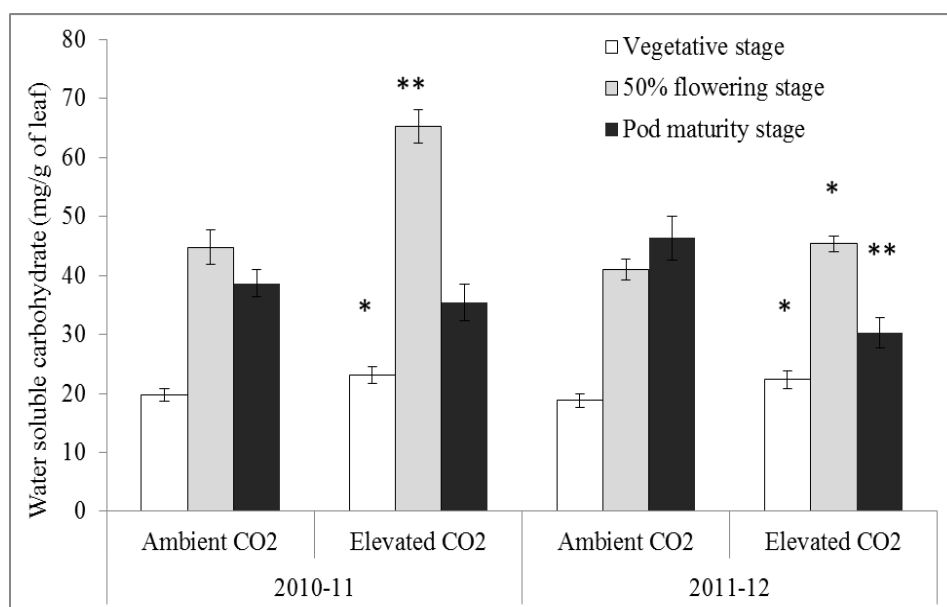
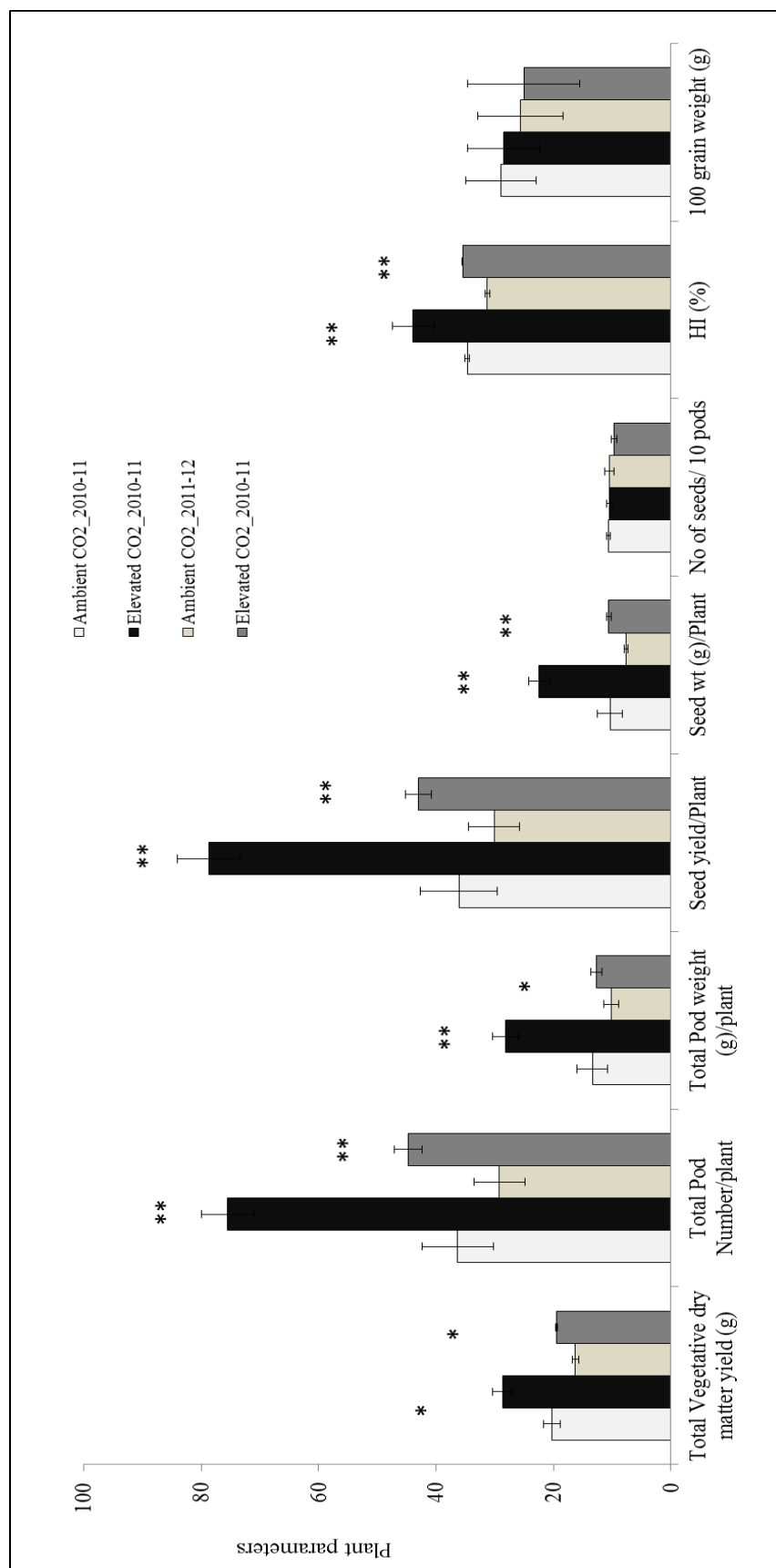


Fig. 5.8: Water soluble carbohydrate content of leaf (per unit weight basis) of chickpea at different crop growth stages under ambient and elevated CO₂ condition during 2010-11 and 2011-12.

Fig. 5.9: Yield component analysis of chickpea at ambient and elevated CO₂ condition for 2010-11 and 2011-12.

* Significance at $p \leq 0.05$ level.

** Significance at $p \leq 0.01$ level.



6. RESEARCH PAPER 3

Resource use efficiency: A function of atmospheric CO₂ enrichment for chickpea (*Cicer arittinum* L.)

Abstract

Open top chamber experiment was conducted to evaluate the change in crop water use in relation to canopy radiation interception under elevated CO₂ for Pusa-1105 chickpea genotypes with the atmospheric CO₂ enrichment at 580±20 ppm level, for two consecutive years 2010-11 and 2011-12 in the research farm of Indian Agricultural Research Institute, New Delhi. Although elevated CO₂ reduced leaf stomatal conductance, reduction in leaf water potential invaded to increase in canopy temperature. Increase in Normalized Difference Water Index (NDWI) values and relative leaf water content also signifies the finding of hindrance in leaf level transpiration that was related to the increase in plant water content, resulted increase in canopy temperature. Besides, significant increase in LAI due CO₂ fertilization effect cannot compensate the reduction in leaf level stomatal conductance. Although water use efficiency (WUE) and radiation use efficiency (RUE) was increased under elevated CO₂ but canopy conductance remained unchanged. Thus our results suggest the findings of improved crop water status under elevated CO₂ with enhanced biomass production. This signifies the possibility of the beneficial effect of atmospheric CO₂ enrichment on chickpea production in dryland agriculture, with its better resource (light and moisture) conversion efficiency.

Keywords: crop water use, water use efficiency, radiation use efficiency, crop conductance, Normalized Difference Water Index, chickpea.

6.1 Introduction

The global atmospheric CO₂ concentration has risen from 280 µmol mol⁻¹ in pre-industrial era (Monastersky, 2013) to 396.71 µmol mol⁻¹ at present over the past 200 years. The rate of increase has accelerated during the last 53 years, increasing from an average of 0.84 µmol mol⁻¹ year⁻¹ in the 1960s to 2.02 µmol mol⁻¹ year⁻¹ in 2000–2012 (<http://www.esrl.noaa.gov/gmd/ccgg/trends/>, accessed 19th July 2013). Increases in greenhouse gas concentrations will be the major cause of global climate change, which could have large impacts on crop production because of possible increases in

abiotic stress. The increasing atmospheric CO₂ concentration in this century has the possibilities to significantly impact crop yield quantity and quality (Long *et al.*, 2006; Ainsworth and McGrath, 2010). The crop water and irrigation use may change as a function of climate change (Lovelli *et al.*, 2010).

Rabha and Uprety (1998) documented decrease in leaf water potential and increase in relative water content in *Brassica*, under elevated CO₂. Increased canopy temperature in potato (Magliulo *et al.*, 2003), sorghum (Triggs *et al.*, 2004) and rice (De Costa *et al.*, 2003; Yoshimoto *et al.*, 2005) was reported in contrast to the findings of decreased IR temperature in pigeon pea (Saha *et al.*, 2012) with increased crop water use. With few exceptions for tree species (Curtis and Wang, 1998), it is a well-established that increasing CO₂ induces stomatal closure in most herbaceous species, regardless of photosynthetic pathway (Kimball and Idso, 1983; Morgan *et al.*, 2004). This can result in reduced transpiration per unit leaf area, and under many circumstances, particularly in canopies that are aerodynamically rough or have low leaf area, lead to reduced canopy evapotranspiration (ET) and higher soil water content compared to non CO₂-enriched plant communities (Field *et al.*, 1995).

Ghannoum *et al.* (2001) reported about the beneficial effect of CO₂ enrichment of 680 ppm due to increase in water-use efficiency (WUE) by offsetting water stress effect in C₄ grasses under drought conditions (Conley *et al.*, 2001) due to increased stomatal resistance (Robredo *et al.*, 2007). Similar increase in the WUE was also reported for wheat (Hunsaker *et al.*, 2000), maize (Chun *et al.*, 2011), barley (Robredo *et al.*, 2007), *Phalaris arundinacea* L (Ge *et al.*, 2011) and pigeon pea (Saha *et al.*, 2011). Although elevated CO₂ increases intrinsic water use efficiency of different crop species, but the magnitude of this effect and its interaction with climate is still poorly understood (Battipaglia *et al.*, 2013). Nelson *et al.* (2004) reported increased water drainage and deep soil moisture storage in the semi-arid grassland ecosystem with increased water use efficiency for several C₃ grasses. Adam *et al.* (2000) reported that under adequate water supply the CO₂ enrichment decreases the stomatal conductance, which allowed plants to decrease transpiration, but photosynthesizing at rates similar to plants growing in ambient CO₂ condition. Lockwood (1999) attributed the increase in water use efficiency the suppressing effect of elevated CO₂ on stomatal conductance as well as transpiration per unit leaf area and the enhancement of carbon assimilation (Robredo *et al.*, 2007). Schapendonk *et*

al. (1997) reported that the total transpiration on a canopy basis was equal for the ambient and doubled atmospheric CO₂ levels. Aranjuelo *et al.* (2005) further supported the fact that elevated CO₂ enhanced water use efficiency because of reduced water consumption and a greater dry mass production in alfalfa.

Regarding crop water use, it was also found to get modified by elevated CO₂. Reduction in cumulative seasonal ET was reported for wheat (Hunsaker *et al.*, 2000), maize (Chun *et al.*, 2011), Potato (Magliulo *et al.*, 2003), sorghum (Conley *et al.*, 2001), soybean (Bernacchi *et al.*, 2007) and model grassland (Grunzweig and Korner, 2001) grown under elevated CO₂. In contrast, Hunsaker *et al.* (1994) and Dugas *et al.* (1994) reported little effect of CO₂ on the cotton crop ET under FACE. Kammann *et al.* (2005) reported no increase of soil moisture in grassland and no change in leaf area index under elevated CO₂. In contrast, supporting the findings of increase in cotton ET (Kimball *et al.*, 1994), greater cumulative crop water use was reported for elevated CO₂ grown pigeon pea throughout the growth period (Saha *et al.*, 2011). Elevated CO₂ also significantly increased canopy remote sensing indices, indicating a stimulation of LAI and photosynthetic carbon assimilation (Gray *et al.*, 2010). Leaky *et al.* (2009) reviewed the suppressing effect of elevated CO₂ on crop water use at both leaf and canopy scale. Bunce and Nasyrov (2012) reported the failure of high CO₂ to protect soybean growth from the soil water stress might be related to the lower hydraulic conductivity of stressed soybeans grown at elevated compared with ambient CO₂.

At the canopy level, CO₂ enrichment was observed to increase LAI (Dermody *et al.*, 2008; Saha *et al.*, 2012) and stimulate increase in RUE due to enhanced growth rates (Long and Drake, 1991; Franzaring *et al.*, 2008). It enabled the crop plants to accumulate more dry matter (Ainsworth and Long, 2005) more efficiently in expense of intercepted canopy radiation, ultimately resulted a net increase in RUE for several cultivated crop species, viz, rice (Weerakoon *et al.*, 2000), wheat (Manderscheid *et al.*, 2003), sunflower (Hui *et al.*, 2001), pigeon pea (Saha *et al.*, 2012). In contrast, De costa *et al.* (2006) found a sharp decrease in rice RUE, under elevated CO₂ during the post-heading period.

One natural concern for future irrigated agriculture is how higher CO₂ concentrations will affect canopy evapotranspiration (ET) and, hence, the irrigation water management of different field crops. Larger and more vigorous plants from a

CO₂-enriched environment may be associated with modified crop ET. Canopy evapotranspiration, integrates the response of stomatal resistance of individual leaves over leaf area expansion factor, leaf surface temperature and changes in developmental stages. However, CO₂ enrichment may also slow leaf transpiration per unit leaf area by partially closing stomata (Ainsworth and Long, 2005; Kirkham, 2011). So, there is a tradeoff between elevated CO₂ induced LAI amplification having the potential to increase evapotranspiration (ET) on one hand and elevated CO₂ induced stomatal enclosure resulting in lower transpiration rates at leaf level on another. Both WUE and its positive interaction with elevated CO₂ were reported to be positively correlated with global irradiance (Schapendonk *et al.*, 1997). The significant increase in stomatal resistance of the most superficial leaves of the canopy leads to a decrease of the vapor transfer to the atmosphere, but at the whole canopy level, increase in LAI due to atmospheric CO₂ fertilization has the potential to offset the increase in individual leaf resistance (Bernacchi *et al.*, 2007). So it is important to understand if role of stomatal resistance in reducing vapor transfer is overshadowed by the increase in LAI under elevated CO₂ and how it modifies crop WUE.

Available literature has reported the effect of elevated CO₂ on water utilization pattern of several crops, mostly for cereals, oilseeds, tubers and closed forest canopy, but for pulse reports are available for only soybean. Chickpea [*Cicer arietinum* L.], being an important legume of drylands has its immense potential to provide nutritional security, to a large population. It is the third most important food legume having a share of 14.6% in global pulse production (FAOSTAT, 2010), grown on 15.3% of the total global pulses area, adding 8.8 million tons to the global food basket. India is the largest producer of chickpea, accounting for 64% of the global chickpea production (Gaur *et al.*, 2010). Therefore, it is important to study the possible changes in water use utilization pattern of this winter-season pulse crop under elevated CO₂. Further it is important to study how water utilization is dependent on radiation utilization in chickpea under elevated CO₂.

6.2 Material Methods

6.2.1 Site description

The open-top chambers (OTCs) experiment were conducted on a fairly leveled topography for Pusa-1105 *kabuli* chickpea genotype, during *rabi* season (mid November- mid April) of 2010-11 and 2011-12, at Indian Agricultural Research Institute, New Delhi, located at 28°35' N latitude, 77°12' E longitude and at an altitude of 228.16 m above mean sea level.

6.2.2 Climate and soil

The site climate was characterized by a typical semi-arid with dry hot summer and mild winters (an average annual rainfall of 710 mm, of which 80% was received during southwest monsoon from July to September). The mean daily maximum and minimum air temperature varies between 10-29 °C and 2-12 °C respectively. The mean daily evaporation (USWB open pan evaporation) was 3.3 mm/day during the crop growth period.

The surface soil (0-30 cm) of the experimental site was non-calcareous and sandy loam in texture with 68.8 % sand 19.2% silt and 12.2% clay. The average bulk density of the soil was 1.61 Mg m⁻³; Water holding capacity, 32.6%; saturated hydraulic conductivity, 1.17 cm h⁻¹; pH (1:2.5 soil: water suspension), 7.9; EC, 0.46 dS m⁻¹; organic C, 0.03 g kg⁻¹; total N, 0.031%; and available P and K, 5.046 and 453.86 kg ha⁻¹, respectively.

6.2.3 Experimental details

The circular structure of OTCs was fixed in the field and fabricated using aluminum frame as described by Saha *et al.* (2011). During the entire experimental period, temperature and humidity inside all four chambers were recorded continuously with the help of sensors (Model TRH 511, Ambetronics, Switzerland) fitted in the middle of each OTC and connected to data logger (Model TC 800D, Ambetronics, Switzerland). In two OTCs, elevated levels of 580 µmol mol⁻¹ CO₂ were maintained from 6:00 AM to 6:00 PM from the crop emergence and the other two OTCs were used as chamber controls without any external supply of CO₂. Regular washing of the

OTC chambers were done using a gentle stream of water to remove the dust and foreign material and to maintain PVC sheet transparency.

6.2.4 Crop Management practices

Seeds of chickpea (*Cicer arittinum* L.; cv. Pusa 1105- *kabuli* type) were treated with the fungicide captan @ 2 g kg⁻¹ of seed. In the next day, seeds were surface sterilized by dipping in 0.1 % HgCl₂ for 2 min. After rinsing in sterile distilled water, seeds were allowed to imbibe water for 6 h and were then coated with *Rhizobium* culture, in addition with 10% sucrose solution in shade. The seed were then sown manually at 10 cm depth by putting 2–3 seed per hole in rows drawn by tyne with a spacing of 30 cm × 15 cm for row-to-row and plant-to-plant, respectively. Basal dose of 20-40-60 NPK was incorporated during seed bed preparation and the sowing operation was completed within second week of November. After 3 weeks, plants were thinned and allowed to grow with a density of 20 plant m⁻² in each open top chamber. Weeding was done manually, while fungicide mixture (Bavistin + Dithane M-45) was sprayed at 50 days after sowing (DAS) to prevent the fungal infection.

6.2.5 Plant sampling at different crop growth stages

To estimate the dry matter partitioning used to calculate the resource use efficiency (radiation use efficiency and water use efficiency, the above ground plant parts were harvested randomly in four replicate from each OTC at three distinct crop growth stages. viz. vegetative, 50% flowering and Pod maturity stage. Based on physical appearance, the specific crop growth stages appeared at 50, 94 and 125 DAS for 2010-11 and 48, 72 and 90 DAS for 2011-12 respectively.

6.2.6 Leaf diffusive resistance

The LI-1600 Steady State Porometer (LICOR Inc., Lincoln NE, USA) was used for rapid and direct readout of diffusive resistance, concurrent measurements of RH of cuvette and leaf temperature. Reading was taken weekly on clear days between 11:30 and 12:00 h in three replicates. As chickpea exhibit a uni-imparipinnate leaves, so porometer readings were taken for the growing branches with alternate clumping of two top most leaves one upon another, shaping them into a continuous leaflet.

6.2.7 Canopy Thermal properties

Hand-held IR thermometer (AG-42, Telatemp Crop. USA) with 8° field of view, was used to measure canopy IR temperature (°C) in four replications for each OTC, at an angle of approximately 45° to the horizontal, in a range of directions such that it shoot plant canopy.

6.2.8 Leaf water potential (ψ)

Tender sun exposed leaves attaining the physiological maturity (3rd to 5th leaf counted from the top and from two plants) were sampled with intact petiole, in four replications from each OTC between 12:30 -1:00 PM and brought to the laboratory in enclosed polythene bags protected from direct sunlight. Midday leaf water potential (ψ) was measured in four replicates during three specific crop growth stages (as mentioned above), using a pressure chamber (s-pms Instruments, New Delhi) following the method of (Scholander *et al.*, 1964).

6.2.8 Relative Leaf Water Content (RLWC)

Sun exposed leaves (3rd and 4th leaf counted from the top and from two plants) were collected with intact petiole, in four replications from each OTC between 12:30 -1:00 PM. The four segments were put in a plastic bag and stored at 0°C until the measurement of the fresh weight (FW). To obtain full turgor, leaf sections were placed in darkness for soaking overnight in vials containing distilled water at room temperature to allowed complete rehydration. The leaf samples were then carefully blotted dry with filter paper and the turgid weight (TW) was recorded. Dry weights (DWs) of the leaf segments were obtained after drying at 105 °C. Relative water content (RWC) was determined using the equation (Barr and Weatherley, 1962),

$$\text{RWC (\%)} = 100[(\text{FW}-\text{DW})/ (\text{TW}-\text{DW})] \quad \dots\dots\dots (6.1)$$

Where, FW, DW and TW are fresh, dry and turgid weights of the sampled leaf, respectively.

6.2.9 Spectral data analysis

Hand held Field Spec™ 3 spectroradiometer having 25° Field of View (FOV), was used for periodic spectral reflectance measurement of chickpea covering the spectral

range between 350 to 2500 nm. The spectral data were processed and exported to Microsoft excel through ASD View Spec Pro software to calculate Normalized Difference Water Index (NDWI) (Chen *et al.*, 2005) as given below:

$$\text{Normalized Difference Water Index (NDWI)} = (R_{858} - R_{1640}) / (R_{858} + R_{1640}) \dots (6.2)$$

Where R is the reflectance at specified wavelength in mm

6.2.10 Leaf area index (LAI)

Leaf-area index was measured at weekly intervals with a plant canopy analyzer (LAI-2000, LI-COR, Lincoln, NE, USA). The LAI was measured at four random locations of each OTC within 1h of sunset when the incident light was diffuse.

6.2.11 Soil moisture (v/v) monitoring

Use of neutron probe for volumetric moisture determination (0-90 cm):

For monitoring soil moisture, neutron probe access tube was permanently installed to a depth of 1.5 m in each of the four OTC at the center. The soil moisture content was monitored at a counting rate of 60s at weekly intervals using the neutron moisture meter (CPN 503 DR HYDROPROBE MOISTURE GAUGE, International INC., USA.) for each 30-cm soil layer increments (15-30, 30-60, 60-90 and 90-120 cm depth) following Zhang *et al.*(2000). Gravimetric soil moisture measurement along with NMM reading in a fallow plot with similar soil, were used to develop separate calibration curve for each soil depth.

The gravimetric soil moisture content was expressed by weight as the ratio of the mass of water present to the dry weight of the soil sample (Black, 1965). Periodic measurement of gravimetric soil moisture content of surface soil (0-15 cm) were used in combination with neutron moisture meter readings from lower depths to study the soil moisture depletion for the entire crop growth period.

Soil moisture depletion rates were calculated and reported as mm day⁻¹. The accumulated depletion was calculated for entire crop growth period. The crop water use was computed using soil water balance equation. Root water uptake during the season was computed from the depletion of soil moisture from 0-90 cm soil. Drainage flux was calculated by subtracting the change of volumetric moisture at 90-120 cm

depth from the relative change of moisture between 0-90 cm depths for a specific time period, assuming no horizontal movement occurred as the soil moisture was very low during this period. Water use efficiency (WUE) of the crop was calculated from the regression between periodic biomass harvested and the amount of water used (cm) over these periods.

6.2.12 Radiation utilization efficiency

Intercepted Photosynthetically Active Radiation (IPAR) by the whole canopy was measured weekly by using apogee point quantum sensor SQ-215 model on clear sunny days between 11:30 - 12:30 hours IST. Eight measurements were averaged for each chamber, four with inter-row placement and the other four with intra-row placement. The fractional interception of PAR (fIPAR) was calculated (Rizzalli *et al.*, 2002) as given below:

$$\text{fIPAR} = (I_0 - I) / I_0 \dots\dots\dots (6.3)$$

Where, I_0 = Incident PAR on the top of the canopy, I = Transmitted PAR at the bottom of the canopy.

The daily incident solar radiation, recorded by pyranometer, was multiplied with a factor of 0.48 to calculate daily incident PAR. The fIPAR calculated for each week was interpolated for each day after sowing and then it was multiplied with daily incident PAR to calculate intercepted PAR. The intercepted PAR was then summed over a specific crop growth period to calculate the cumulative intercepted radiation, which was further corrected for 85% transmittance of sunlight through the polythene cover of OTC. Radiation use efficiency (RUE) was calculated as the slope of the plotted linear relationship between total biomass accumulation for a specific crop period and cumulative intercepted radiation during that period and expressed in g MJ^{-1} unit.

6.2.13 Calculation of crop conductance (g_c)

At the crop level, radiation-use efficiency (RUE) is proportional to water-use efficiency (WUE) in terms of total dry matter accumulation (Sadras *et al.*, 1991). The relation can be modeled as:

$$\text{RUE} = \text{WUE} \times g_c \dots\dots\dots (6.4)$$

where, g_c is the ET per unit intercepted radiation i.e. crop conductance (Sadras *et al.*, 1991; Caviglia and Sadras, 2001). We have evaluated the value of g_c for chickpea under elevated and ambient CO₂ at specific time intervals for the entire crop growth periods during both of the years of our experimentation likely,

$$g_c = \text{RUE} / \text{WUE}$$

$$= (\text{Biomass produced} / \Sigma \text{IPAR}) / (\text{Biomass produced} / \Sigma \text{Crop water use})$$

$$= (\Sigma \text{Crop water use} / \Sigma \text{IPAR}) \dots\dots\dots (6.5)$$

6.2.14 Statistical analysis:

Statistical analysis of the data was done by two sample t test (two-tailed hypothesis) using SPSS statistical software Version 16.0 (SPSS Inc., Chicago). Zar-t test (Zar, 2010) was used to test significant difference between two slopes values.

6.3 Results

Elevated CO₂ resulted in increasing of stomatal diffusive resistance of chickpea leaves (Fig. 6.1). The increase over control was prominent after 64 DAS till maturity, ranging from 6.8% ($p < 0.05$) to 40.1% ($p < 0.01$) during 2010-11. In the second year, the similar trend was observed after 67 DAS but the magnitude of increase was comparatively less varying between 2.5% to 26.5% ($p < 0.05$).

Mid-day leaf water potential was measured at different phenological stages by destructive sampling showed a significant increasing trend under elevated CO₂ (Fig. 6.2a). At vegetative crop growth stage, there was no significant difference in leaf water potential between elevated and ambient CO₂ treatments during 2010-11, but during 2011-12 it was increased by 26.6% ($p < 0.01$) under elevated CO₂. Afterwards the difference increased further and reached maximum at 50% flowering stage with a value of 84.7% ($p < 0.01$) and 66% ($p < 0.01$) during 2010-11 and 2011-12, respectively. At pod maturity the difference slightly narrowed down to 53.6% ($p < 0.01$) and 43.1% ($p < 0.01$) during 2010-11 and 2011-12, respectively.

Relative plant leaf water content it also showed a similar trend of increase under elevated CO₂ (Fig. 6.2b). Although during 2010-11, there was no significant effect of elevated CO₂ on the value of relative leaf water content at vegetative stage

but at 50% flowering and pod maturity it was found to be higher by 7% ($p < 0.01$) and 9% ($p < 0.05$) respectively. During 2011-12, the relative water content was found to be higher by 7.3% ($p < 0.05$) at vegetative stage and about 9.5% ($p < 0.05$) at both 50% flowering and reproductive stage, under elevated CO_2 treatment.

Canopy IR temperature was higher under elevated CO_2 for than that of ambient CO_2 , during both of the years of experimentation (Fig. 6.3). The temperature difference between elevated and ambient CO_2 treatment widened between 60 to 100 DAS, mostly around the 50% flowering stage in both of the years of experimentation.. It was observed to be 1.24°C ($p < 0.01$) and 1.7°C ($p < 0.01$) higher during the two consecutive years. On an average the canopy IR temperature was about 0.86°C higher for elevated CO_2 treatment in 2010-11, which was amplified to 1.3°C in the next year over the whole crop growth duration. The canopy IR temperature for both the treatments in two years showed a typical pattern of first decreasing with crop growth till 55 DAS, then remained low around $20\text{--}25^\circ\text{C}$ and then increasing again towards crop maturity (Fig. 6.3).

Normalized Difference Water Index (NDWI), a remote sensing index indicating canopy moisture status was significantly under elevated CO_2 during both of the years of the experimentation (Fig. 6.4). During 2010-11, it was found to be about 24% ($p < 0.01$) higher at vegetative stage and the difference increased to 48% ($p < 0.01$) at 50% flowering stage. After 120 DAS, when the crop was moving towards its maturity, the difference narrowed down to 11.2%, which was not statistically significant. During 2011-12, the difference between elevated and ambient CO_2 treatment was found to be 38.1% ($p < 0.05$) at vegetative stage, which was reduced to 26.6% ($p < 0.01$) at 50% flowering. But at pod maturity, the magnitude of increase in NDWI values was reduced to 9.2% which also was not statistically significant. The NDWI results indicate again higher canopy moisture content under elevated CO_2 .

Leaf Area Index increased due to elevated CO_2 fertilization effect. During 2010-11, the canopy LAI was significantly higher ($p < 0.01$) from 45 DAS till crop maturity for plants grown under elevated CO_2 than control (Fig. 6.5). As compared to ambient CO_2 treatment, peak LAI value was found to be 22.6% ($p < 0.01$) higher under elevated CO_2 at 111 DAS (post flowering stage) and thereafter it declined

gradually as the crop matured. During the second season, the CO₂ induced amplification of crop LAI started to dominate 30 days after sowing. The value attained by the second year crop was 21.3% ($p < 0.05$) higher under elevated CO₂. Thereafter, it was declined steadily till crop maturity.

Cumulative crop water use was computed from soil moisture depletion data from 0-90 cm depth over the entire crop growth period considering ET as major component of water loss (Fig. 6.6). During 2010-11, elevated CO₂ exposed chickpea plants depleted 39.7 cm of water as compare to 43.8 cm for plants grown under ambient CO₂. In the second year 2011-12, the elevated CO₂ exposed plants depleted a total 20.3 cm of water as compared to 22.4 cm for ambient CO₂ exposed plants. In both the years the cumulative water use was found to be lower under elevated CO₂ than control but the difference was not statistically significant at $p < 0.05$. It indicates that cumulative water use of chickpea is not affected significantly under elevated CO₂.

Crop water use efficiency was calculated as slope of linear regression line between biomass and cumulative water use with no intercept by pooling treatment wise data of two years of experimentation (Fig.6.7a). The average WUE was 22.25 g m⁻²cm⁻¹ ($R^2 = 0.71$, $p < 0.05$) under elevated CO₂ and was 8.69 g m⁻²cm⁻¹ ($R^2 = 0.81$, $p < 0.05$) under ambient CO₂ grown plants. Increase in WUE of chickpea was about three times under elevated CO₂ as compared to control.

Two year's data of above ground biomass and cumulative intercepted PAR was pooled to derive the RUE. The slope of linear regression with zero intercept between above ground biomass and cumulative intercepted PAR gave the RUE (Fig. 6.7b). The RUE value was 0.313 g MJ⁻¹ for elevated CO₂ as compared to 0.133 g MJ⁻¹ under ambient CO₂ with R^2 values of 0.80 ($p < 0.05$) and 0.88 ($p < 0.05$), respectively. The results indicate that elevated CO₂ treatment results in significant increase in chickpea RUE. The Zar-t value showed that the RUE value was 135.3% higher ($p \leq 0.05$) under elevated CO₂ than under control. The increase in above ground biomass under elevated CO₂ was the major factor contributing to increase in crop RUE.

Crop conductance (g_c) was calculated as slope of linear regression line between cumulative water use and cumulative IPAR with no intercept by pooling treatment wise data of two years (Fig. 6.8). The g_c was 0.14 mm MJ^{-1} ($R^2 = 0.88$, $p < 0.01$) and 0.15 mm MJ^{-1} ($R^2 = 0.90$, $p < 0.01$) under elevated and ambient CO_2 respectively. The difference was statistically non-significant. The results indicate that chickpea continue to use same amount of water per unit of radiation interception even under elevated CO_2 , even though there is a significant increase in WUE and RUE.

Total plant biomass produced under elevated CO_2 was significantly higher for both of the years of our experimentation (Fig. 6.9). At vegetative stage, the increase in total plant biomass was 1.83 times ($p < 0.05$) and 2.23 times ($p < 0.05$) under elevated CO_2 during the two consecutive years of experimentation. Similar trend was maintained at 50% flowering stage with an increase of 1.67 times ($p < 0.01$) and 1.72 times ($p < 0.01$) in two years, respectively. But, after pod formation the difference was amplified to 2.3 times ($p < 0.01$) for 2010-11 and 2.1 times ($p < 0.01$) for 2011-12 at harvesting maturity.

6.4 Discussion

Plants influence ecosystem water balance through their physiological, phenological, and biophysical responses to variable environmental conditions. In support of the findings of increased stomatal resistance under elevated CO_2 of $700 \mu\text{mol mol}^{-1}$ (Li *et al.*, 2004a; Robredo *et al.*, 2007), we also observed similar trend of increased average mid-day diffusive resistance (Fig. 6.1) for chickpea by 16.2% ($p < 0.05$) under atmospheric CO_2 enrichment, during the entire crop growth period in both of the years of our experimentation. This increase in resistance reduces transpiration per unit leaf area (Kimball *et al.*, 2002; Li *et al.*, 2003). Our findings suggest that with increase in atmospheric CO_2 concentration crops should display ever-increasing reductions in transpirational water loss due to partial stomatal closure (Kirkham, 2011). Brearley *et al.* (1997) suggested the role of channel regulation process in the guard cells under elevated CO_2 to control both anion and K^+ channels to achieve net solute loss in cytosolic pH. CO_2 elevation was also reported to increase stomatal resistance by triggering the increase in cytoplasmic free calcium (Vavasseur and Raghavendra,

2005) that was not associated with a similar change in stomatal density (Ainsworth and Rogers, 2007).

In a better agreement with Wullschlegel *et al.* (2002b) and Nelson *et al.* (2004), we observed the elevated CO₂ exposed chickpea plants maintained higher (less negative) leaf water potentials (Fig. 6.2a) during most of the crop growth stages along with similar findings of increased relative leaf water content (Fig. 6.2b) as reported for short grass steppe (Morgan *et al.*, 2001) and maize (Meibaum *et al.*, 2012). This increase in leaf water content was associated with increment in stomatal resistance. The high CO₂-induced stomatal closure may results into increased leaf water potential due to the changes in plant structure, i.e. vessel or tracheid anatomy, leaf specific conductivity (Tyree and Alexander, 1993).

Following an initial period of uncertainty, due to greater presence of soil in the optical view of the instrument (up to first 60 DAS), consistent higher canopy IR temperature (Fig. 6.3) was observed under elevated CO₂ during both of the years of experimentation. This effect again is related to stomatal closure in response to the increase in CO₂ partial pressure (Triggs *et al.*, 2004), which reduces plant transpiration per unit leaf area, and thus the cooling effect (Kimball *et al.*, 1993; Magliulo *et al.*, 2003).

NDWI was used to evaluate the canopy water content (Fig. 6.4) at different time phase of canopy development. NDWI is sensitive to changes in liquid water content of vegetation canopies. The variation in NDWI showed a significant higher value in both of the years of experimentation suggesting that canopy exposed to atmospheric CO₂ enrichment bears more water in the leaf tissues (Gao *et al.*, 1996). NDWI is also an indirect estimation of canopy condition, due to its close association with NDVI (Chen *et al.*, 2005). Thus the higher NDWI was also an indirect indicator of good plant vigour resulting from greater level of photosynthetic activity (Peters *et al.*, 2002) for the plants grown under elevated CO₂. Higher LAI also strengthens this fact (Gamon *et al.*, 1995).

The LAI growth profiles observed in this study under ambient and elevated CO₂ are typical for any crop in which LAI increases during vegetative growth, then saturating at flower initiation and declining during senescence (Fig. 6.5). During

2011-12, the LAI was lower throughout the growth period than during 2010-11 on account of late sowing by about one month (Haloi and Baldev, 1986). The increase in LAI of chickpea under elevated CO₂ is clearly a manifestation of CO₂ fertilization affect (Kim *et al.*, 2001), where plants produce more biomass due to higher availability of carbon and absence of any other resource's constraint. Similar findings have been reported by Daepf *et al.* (2001) for *Lolium perenne* and Oikawa *et al.* (2013) for soybean crop. The effect of elevated CO₂ was most dominant at peak LAI growth stage which increased by 22.6% ($p < 0.01$) in 2010-11 and 41% ($p < 0.01$) in 2011-12 over control. Our result was supported by the findings of elevated CO₂ induced increase in maximum LAI in soybean (Dermody *et al.*, 2008) and pigeon pea (Saha *et al.*, 2012).

Regarding crop water use we observed no significant difference in cumulative crop water use for 2010-11 ($p = 0.586$) and 2011-12 ($p = 0.808$) under elevated CO₂ as compared to control (Fig. 6.6). During 2010-11, the relative increase in per day crop water use after 98 DAS in elevated CO₂ environment may possibly be attributed to the over shadowing effect of canopy LAI amplification (Shimono *et al.*, 2013). The peak LAI was observed to be amplified by 22.6% ($p < 0.05$) at 94 DAS, overshadowing the elevated CO₂ mediated increase in leaf stomatal resistance by 9.5% ($p < 0.05$) and this was maintained till pod maturity stage. But in the second year of experimentation although the LAI was amplified by 21.3% reaching its peak at 80 DAS, but its rapid fall at pod development and maturity stage possibly enabled the relative dominancy of increased stomatal resistance over LAI. As a result no significant change in cumulative crop water use was observed for that year. Chaudhuri *et al.* (1990) grew winter wheat in CO₂-enriched greenhouses for three years and found that although evapotranspiration (ET) increased by 16% at elevated CO₂ (825 $\mu\text{mol mol}^{-1}$) in one year, there was little effect of CO₂ on ET in the other two years. Kimball *et al.* (1994) reported a 13% increase in ET of cotton in the CO₂ enriched plots (550 $\mu\text{mol mol}^{-1}$) compared to ambient conditions in a free-air CO₂ enrichment (FACE) experiment. Thus, our results make a better agreement with Li *et al.* (2004a) suggesting the possibility of CO₂-enrichment-induced decrease in leaf transpiration was almost compensated for the increase in ET brought by the higher leaf area under well-watered and adequate soil N availability in chickpea. Being a

legume, chickpea itself can fix atmospheric N and adequate water supply was also ensured for our experiment as per recommendation for chickpea (Khanna-Chopra and Sinha, 1987). Elevated CO₂ induced increase in stomatal resistance has its least influence in cumulative crop water use (Hui *et al.*, 2001) when LAI get increased due to CO₂ fertilization effect (Saha *et al.*, 2012). At the end of the crop season, we observed no significant difference in cumulative crop water use integrated over the entire crop growth period during both the years of our experiment but with an average increase in crop water use efficiency by about 2.56 times ($p < 0.05$) for chickpea (Fig. 6.7a). This increase in water use efficiency of chickpea was mainly attributed to significant increase in total plant biomass at different crop growth stages due to atmospheric CO₂ fertilization effect (Fig. 6.9). Although the increase in total plant biomass was maintained under elevated CO₂ over all the crop growth stages, but the difference was widened after pod formation due to elevated CO₂ induced increase in plant economic produce.

Thus our result revealed the similarity to findings of Adam *et al.* (2000) and Qiao *et al.* (2010), suggesting that no water saving due to elevated CO₂ induced, Although WUE was increased in support of the findings for other C₃ crop likely, wheat (Hunsaker *et al.*, 2000), barley (Robredo *et al.*, 2007), pigeon pea (Saha *et al.*, 2011). De Costa *et al.* (2003) suggested canopy stomatal conductance and daily total canopy transpiration rate of the elevated CO₂ treatment were approximately the same as those achieved under ambient conditions was due to significantly greater LAI and greater leaf–air vapour pressure deficit under CO₂ enrichment.

Improvement in photosynthetic RUE is one of the promising alternatives to increase crop productivity in future and this study showed the potential beneficial effect of elevated CO₂ in enhancing chickpea RUE (Fig. 6.7b). Reduction in dry matter production with reduced radiation interception had been reported in chickpeas (Verghis *et al.*, 1999). The regressed value of RUE of 0.133 g MJ⁻¹ under ambient CO₂ estimated in our study was much lower than any other RUE value reported in literatures for chickpea (0.30-0.93 g MJ⁻¹ by Hughes *et al.*, 1987; 1.4 g MJ⁻¹ by Leach and Beech, 1988; 1.0 g MJ⁻¹ by Soltani *et al.*, 2006; 0.25-0.87 by Jahansooz *et al.* 2007). As in most of the studies reported in literature for different crops, a significant increase in RUE (2.35 times) due to elevated CO₂ was observed in

chickpea also. The increase in RUE may be due to higher biomass production per unit of radiation interception as seen by increase in LAI for most part of growth season. It may also be due to more vertical and longer branches and internodes that improves light distribution within the canopy with reduced PAR interception in the upper canopy (Sims *et al.*, 1999) while increasing PAR interception in the lower canopy resulting higher photosynthetic efficiency (Walcroft *et al.*, 2005) and thus enhanced RUE.

Crop growth stage specific calculation of RUE and WUE also showed significant CO₂ fertilization effect (Fig. 6.8). Although both RUE and WUE were positively influenced by elevated CO₂, but no effect on crop conductance was observed. It indicates that there was no change in crop water use per unit of radiation intercepted under elevated CO₂. It was in contrast to the report of Burkart *et al.* (2004) about the reduction in canopy conductance under elevated CO₂ for well watered wheat. In a close similarity to the available literatures, the increase in crop RUE (Manderscheid *et al.*, 2003; De costa *et al.*, 2006) and WUE (Qiao *et al.*, 2010) in our experiment, could be explained by the elevated CO₂ stimulated enhanced photosynthetic assimilation rates (Ainsworth and Long, 2005) of the crop canopy associated with higher LAI values (Dermody *et al.*, 2008; Saha *et al.*, 2012).

6.5 Conclusions

Although plant efficiency was increased under elevated CO₂ exposure due to increase in terms of better dry matter production but increase in canopy LAI compensate the decrease plant water uptake mediated by elevated CO₂ induced increase in leaf diffusive resistance for chickpea. Stability in crop conductance value under elevated CO₂, with increased RUE and WUE signifies the increase in chickpea plant efficiency to convert both intercepted radiation (PAR) and water increased in equal magnitude, under elevated CO₂, as compare to control. Thus, being a dryland legume the elevated CO₂ plays its beneficial role for chickpea. Ultimately, it resulted into higher biomass production utilizing the similar amount of resources (radiation and water) as available today, but with a greater efficiency for atmospheric CO₂ enrichment condition as predicted in future.

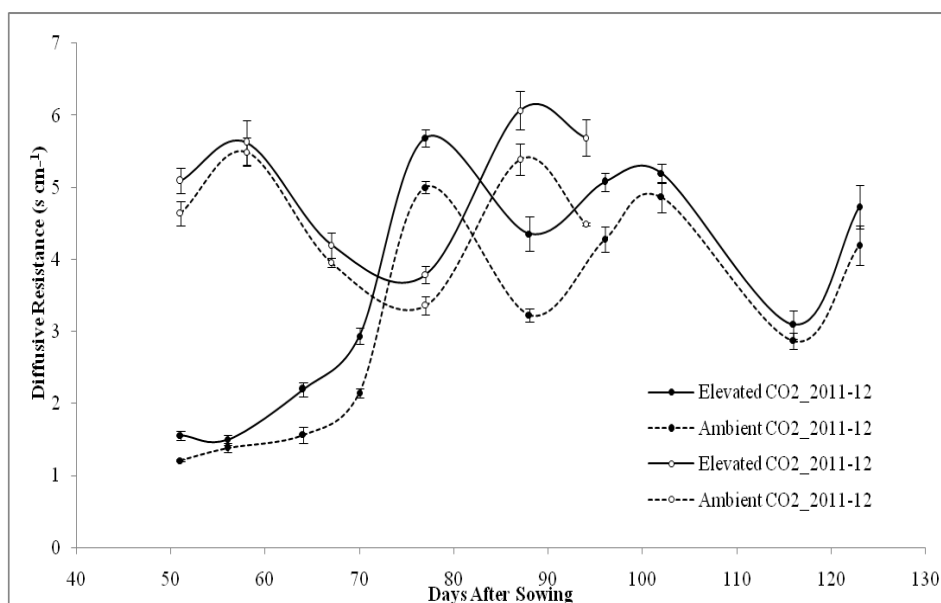


Fig. 6.1: Variation of leaf (a) diffusive resistance in chickpea during the entire crop growth period under elevated and Ambient CO₂ treatment during 2010-11 and 2011-12

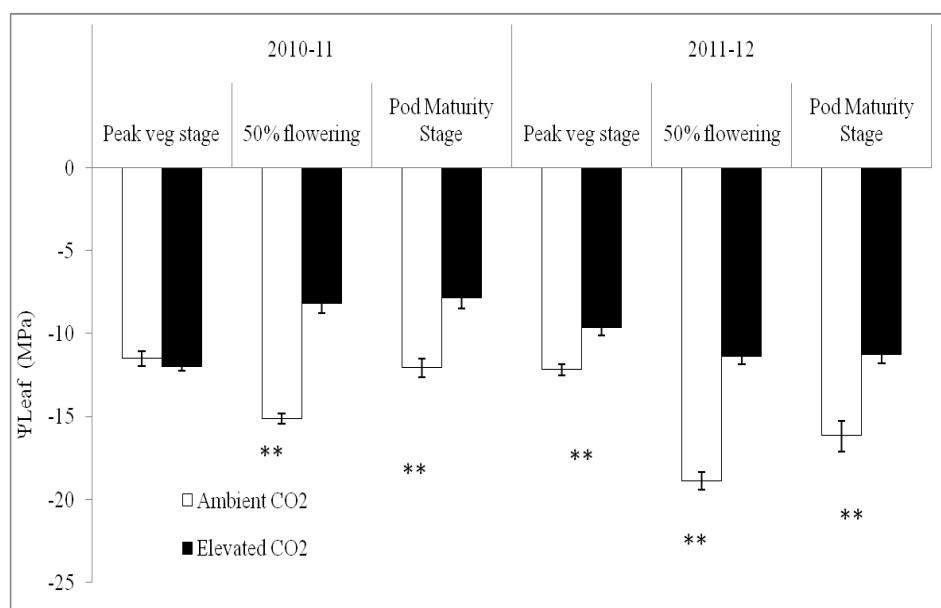


Fig. 6.2a: Variation in leaf water potential in chickpea at different crop growth stages during 2010-11 and 2011-12

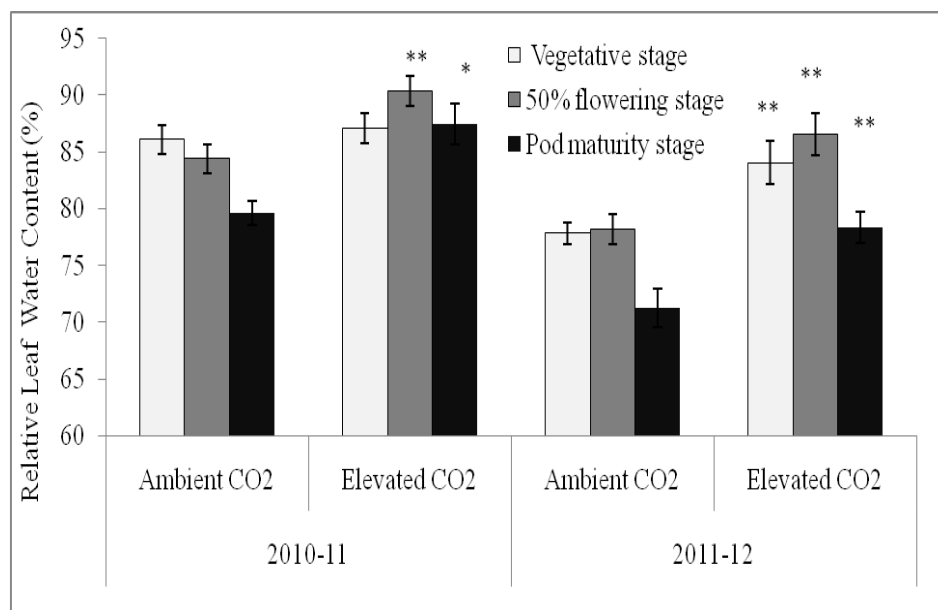


Fig. 6.2b: Variation in relative leaf water content of chickpea leaves at different crop growth stages during 2010-11 and 2011-12

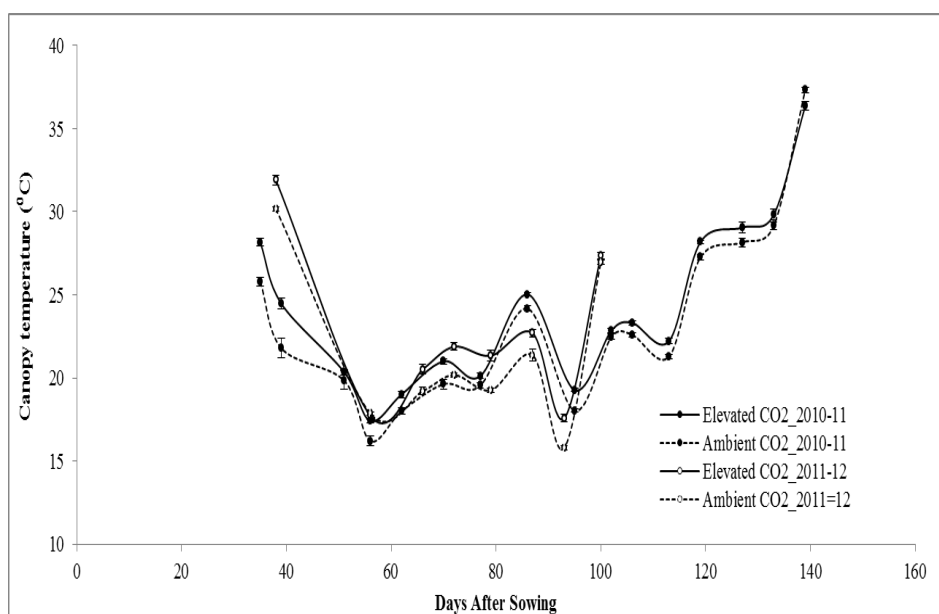


Fig. 6.3: Variation in canopy IR temperature in chickpea for the entire crop growth period during 2010-11 and 2011-12

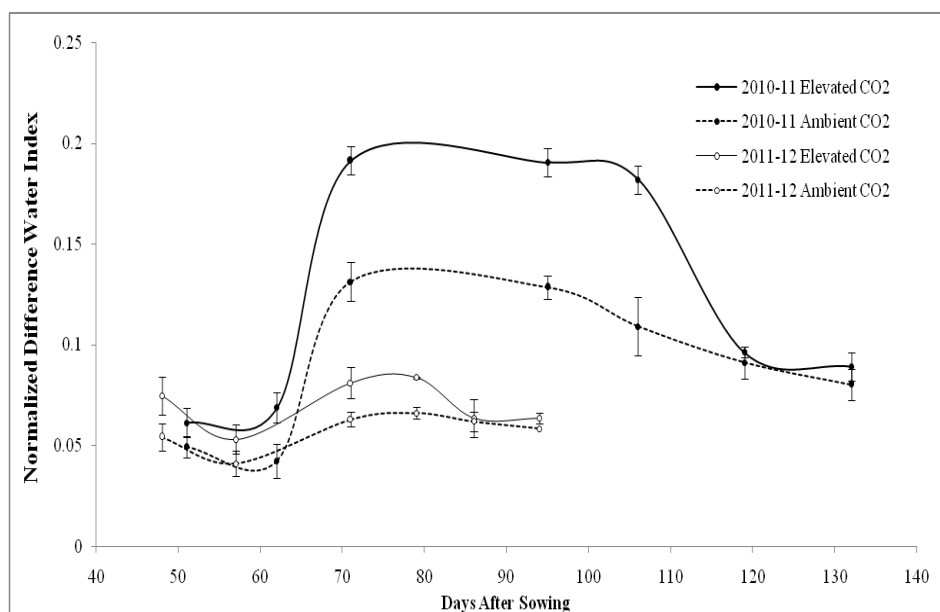


Fig. 6.4: Variation in NDWI for chickpea for the entire crop growth period during 2010-11 and 2011-12

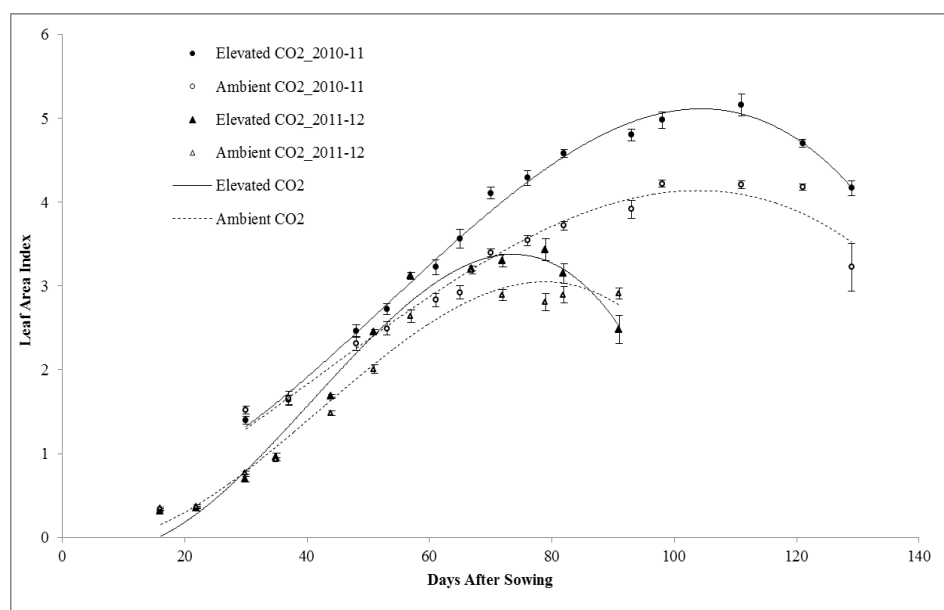


Fig. 6.5: Variation in canopy LAI in chickpea for the entire crop growth period during 2010-11 and 2011-12

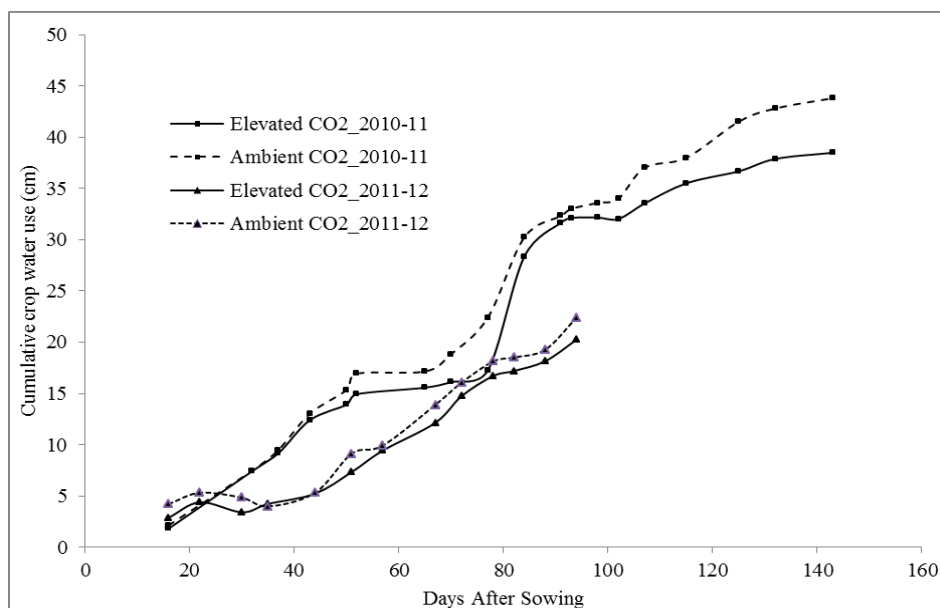


Fig. 6.6: Variation in cumulative crop water use for chickpea for the entire crop growth period during 2010-11 and 2011-12

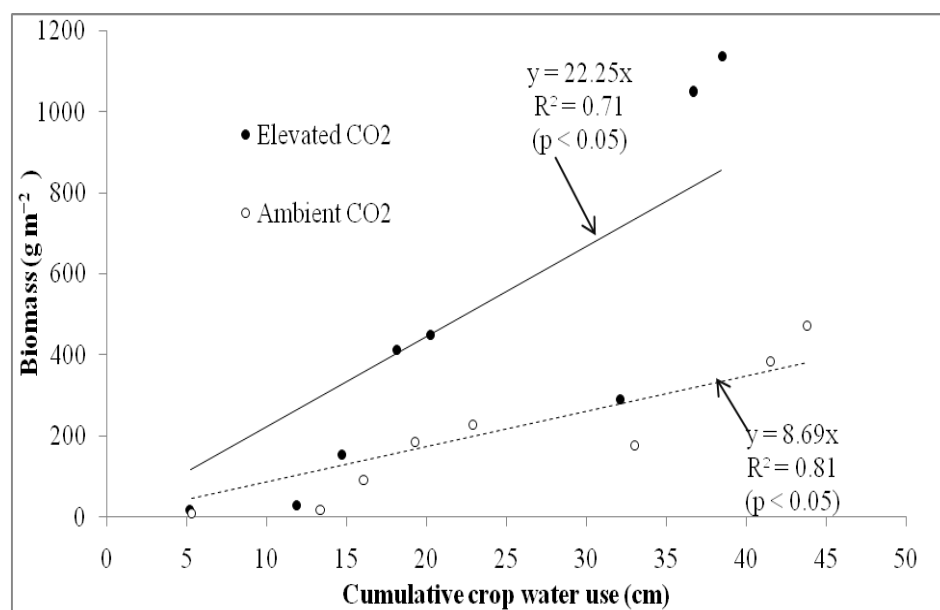


Fig. 6.7a: Relationship between crop water use and biomass produced under elevated and ambient atmospheric CO_2 level in chickpea.

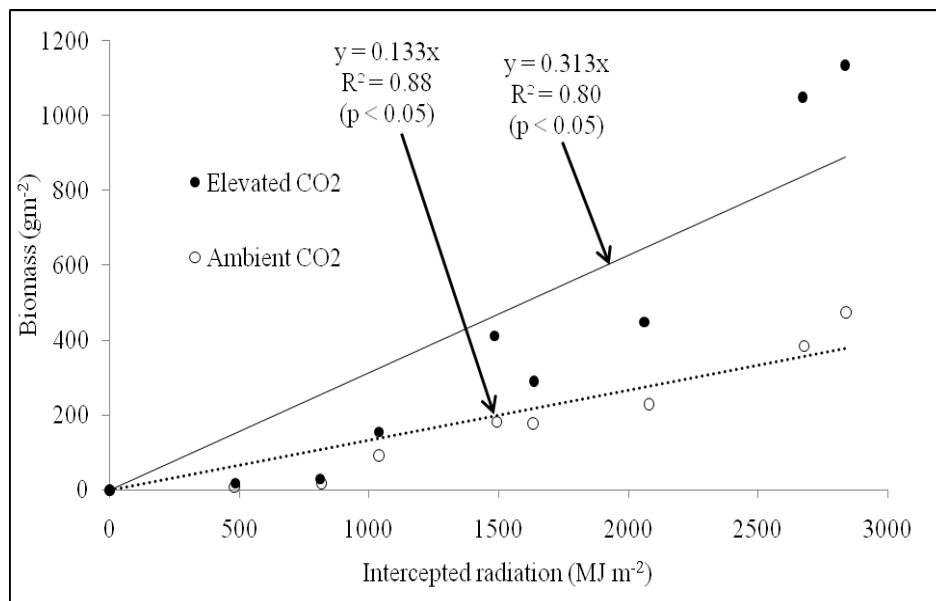


Fig. 6.7b: Aboveground biomass of chickpea as a linear function of cumulative IPAR for different crop growth stages under ambient and elevated CO₂ condition

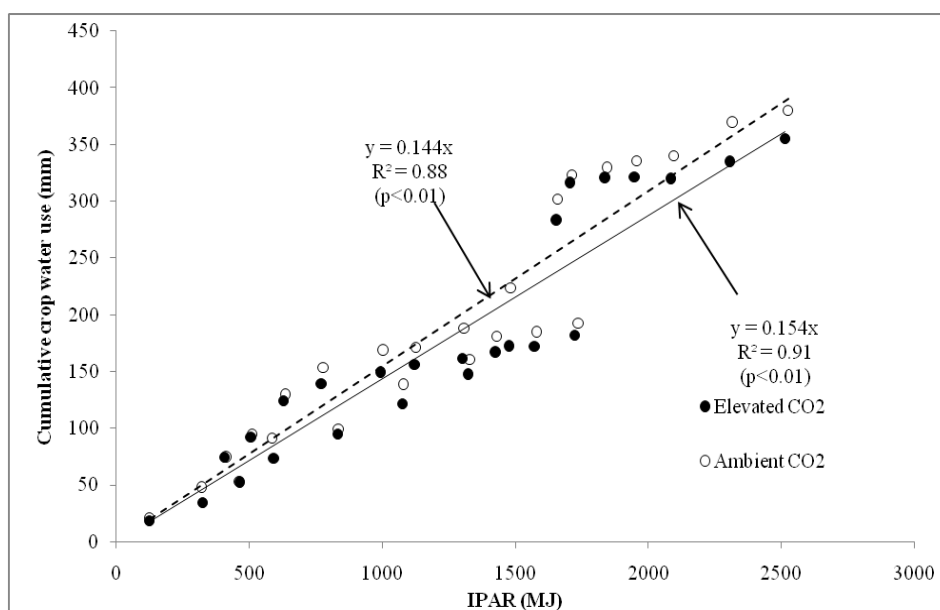


Fig. 6.8: Relationship between cumulative crop water use and intercepted PAR as influenced by elevated CO₂ levels in chickpea.

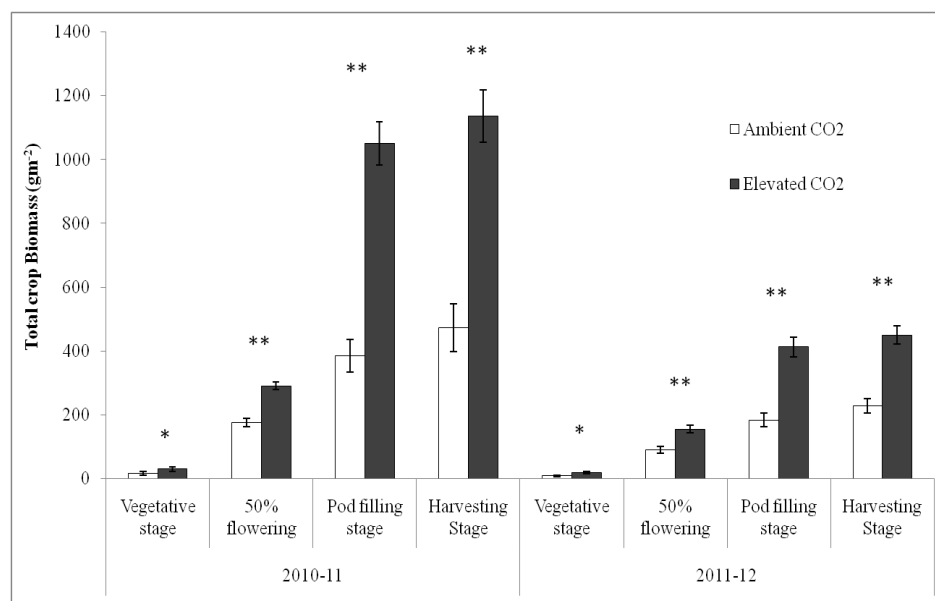


Fig. 6.9: Total crop biomass production (g m⁻²) in chickpea as influenced by elevated CO₂ levels.

7. RESEARCH PAPER 4

Elevated atmospheric CO₂ impacts on rhizosphere carbon and enzyme activities, and root nodule biophysics in chickpea (*Cicer arietinum* L.) crop

Abstract

Impact of six years atmospheric CO₂ enrichment (580±20 µmol mol⁻¹) on soil carbon and enzyme activities in relation to root and nodule biophysics in chickpea was assessed in an open top chamber experiment during two consecutive years (2010-11 and 2011-12). Soil carbon and phosphorus pools showed variable response with associated enzyme activity, but no change in total soil N was recorded. Most of carbon pools did not show any significant change, although decreasing trend in soil recalcitrant C pools was observed. This contributed to concurrent increase in labile pools including water soluble carbohydrate and microbial biomass carbon. Dehydrogenase enzyme activity in soil showed 1.5-fold increase along with 1.8-fold increase in FDA hydrolysis under elevated CO₂. An increase (20%) in soil β glucosidase activity with 48% increase in MBC may be responsible for 1.3 times increase in soil CO₂ flux under CO₂ enrichment. Significant effect of CO₂ enrichment on growth and development of chickpea roots was recorded along with greater N₂ fixation as evidenced by more than 93% increase in leghaemoglobin content and 65% increase nitrogenase activity in nodules. However, C:N ratio in rhizosphere soil was preserved. Nodules tend to be bulky under elevated CO₂ and both the starch and soluble sugar content increased, allowing additional N₂ fixation. Yield and harvest index was higher by 26.5% and 9.85%, respectively. Although there could be a greater potential of increased N₂ fixation by nodules under CO₂ enrichment, a net depletion of soil recalcitrant C pools and increase in labile C fraction might offset C sequestration potential in legume-based system in the semi-arid climate regions.

Key words: Rhizosphere, enzyme activities, soil carbon, root nodules, chickpea, elevated CO₂

7.1 Introduction

Increase in atmospheric CO₂ level is likely to have variable and complex impact on soil ecosystems vis-à-vis global biogeochemical cycles (Janus *et al.*, 2005). Short-

term responses (<10 years) from field experiments may not be adequate to predict the biogeochemical feedbacks and interactions which often occurs at a larger time-scale. Studies exploring long-term responses towards atmospheric enrichment have gained widespread attention in last few decades (Pendall *et al.*, 2004a; van Kessel *et al.*, 2006).

Soil carries the most stable and largest terrestrial C pool, accounting to nearly 80% of global terrestrial C (Nielsen *et al.*, 2011); the capacity depends on the balance between rate of biomass C inputs and C released (as CO₂) through microbial activities. Elevated CO₂ is expected to modify the rates of formation and decay of soil organic C, giving substantial feedbacks to atmospheric CO₂ concentration.

Decomposition of plant materials is controlled by N availability in soils under elevated CO₂ (Pendall *et al.*, 2004b; Viswanath *et al.*, 2010), although short term stimulation of initial C mineralization (Bazot *et al.*, 2006; Hagedorn and Machwitz, 2007) with increase in potentially mineralizable C (Williams *et al.*, 2004) were reported. Meta-analyses indicate that elevated CO₂ can produce small yet measurable increase in soil C storage (Jastrow *et al.*, 2005; Luo *et al.*, 2006). Contrary results do exist, and C content remains unaffected in nutrient-poor native terrestrial ecosystems (De Graaff *et al.*, 2006). Although some studies have revealed that elevated CO₂ had no effect on soil C stocks in grasslands after a decade (Van Kessel *et al.*, 2006; Bazot *et al.*, 2006) and after 4 years (Guenet *et al.*, 2012), a 9-year Free-Air-CO₂-Enrichment (FACE) experiment (Lichter *et al.*, 2008) reported marginal but significant increase in soil C and N content. No significant difference in the C:N ratio was also reported (Anderson *et al.*, 2011). In tropical rice soils, elevated CO₂ increased readily available C content, with enhanced N₂ fixation and thereby higher available N (Rogers *et al.*, 2006; Das *et al.*, 2011). A simulation study indicated positive correlation between soil C stock and CO₂ enrichment for all the vegetation covers (Lin and Zhang, 2012). In another meta-analysis, however, a lower potential for increase in soil C content with increase in root biomass under elevated CO₂ was reported (Sillen and Dieleman, 2012). With few exceptions, soil and microbial respiration rates were higher under elevated CO₂ (Moscatelli *et al.*, 2005) indicating additional substrate metabolism due to larger soil C inputs through enhanced plant growth (Donald *et al.*, 2000). Increase in below-ground plant productivity and metabolism with increased atmospheric CO₂ was reported, suggesting that a

significant additional C is transferred to root systems, and ultimately to the soil microbial community and C pools (Pendall *et al.*, 2004a; van Groenigen *et al.*, 2006; Hill *et al.*, 2007). Microbial and rhizosphere respiration under elevated CO₂ increased (Phillips *et al.*, 1998; George *et al.*, 2003) due to increase in biomass rather than activity of soil microbes, with greater potential to degrade plant materials (Sowerby *et al.*, 2000). However, a number of studies indicated no change in microbial biomass C under elevated CO₂ (Kandeler *et al.*, 2006; Carney *et al.*, 2007; Lesaulnier *et al.*, 2008; Kassem *et al.*, 2008; Anderson *et al.*, 2011; Manna *et al.*, 2013). Elevated CO₂ increased soil respiration (due to increasing root respiration) and enhanced priming of soil organic matter, which may result in ecosystem C losses (Carney *et al.*, 2007).

Soil enzymes reflect the change in population dynamics and activity of soil biota, although little information is available on its response to atmospheric CO₂ enrichment. Elevated CO₂ did not significantly change β -glucosidase and phosphatase activities in bulk soil after 5 years but decreased their activities in microaggregates. However, substrate addition significantly increased both the enzymes activity resulting from accelerated C turnover in soil under elevated CO₂ (Dorodnikov *et al.*, 2009). Increasing trend of soil β -glucosidase activity was also reported elsewhere (Moscatelli *et al.* 2005; Finzi *et al.*, 2006). Xuexia *et al.* (2006) found increase in β -glucosidase activity only under higher N fertilization in a rice-wheat rotation.

Increase in dehydrogenase activity in grass monocultures growing at elevated CO₂, with an associated increased SOM decomposition rate was reported (Dhillon *et al.*, 1996). Similar effect was also reported in oak (Li *et al.*, 2010), rice (Das *et al.*, 2011) and pigeon pea (Saha *et al.*, 2011). However, insensitivity of elevated CO₂ to dehydrogenase was also reported in rice (Manna *et al.*, 2013).

With limited supply of phosphorus, elevated CO₂ increased wheat root phosphatase activity (Ebersberger *et al.*, 2003). A sharp and significant increase in alkaline phosphatase activity in grassland soils due to CO₂ enrichment resulting from higher soil moisture and increased root biomass with subsequent stimulation of N-mineralization was also reported (Kandeler *et al.*, 2006; Lichter *et al.*, 2008). Increase in phosphatase activity of grassland soil was reported by others (Xuexia *et al.*, 2006; Guenet *et al.*, 2012). No change in acid phosphates activity was reported in subalpine forest (Ruifang *et al.*, 2007) and rice (Manna *et al.*, 2013). The increased phosphatase activity is a root mediated response (Barrett *et al.*, 1998), but originates from root

cells rather than root-associated microorganisms (Moscatelli *et al.*, 2005). However, at low P availability, limited response of biomass production to elevated CO₂ may constrain any ecosystem response (Edwards *et al.*, 2006) as root growth and soil C pools were enhanced with more biological N fixation only with high P supply (Lam *et al.*, 2012b).

Roots contribute more to the soil organic C pool than surface residues do (Johnson *et al.*, 2007). Elevated CO₂ has substantial impacts on roots causing increase in root length, diameter and biomass, stele diameter, cortex width, shoot and root weight ratios, but no increase in root numbers (Rogers *et al.*, 1992). Increase root biomass in grasslands (Niklaus *et al.*, 2001; LeCain *et al.*, 2006), length and weight of roots and root: shoot ratios in black gram (Vanaja *et al.*, 2007), root weight, volume, and root: shoot ratio in tomato due to NO accumulation (Wang *et al.*, 2009; 2013) were also reported. Long-term FACE experiment did not show any significant effect of elevated CO₂ on total and fine root biomass and root mass fraction in Poaceae, but showed a significant decrease of 28% in root mass fraction in forbs (Crous *et al.*, 2010). Elevated CO₂ was reported to significantly increase lateral root number, root length, surface area, diameter and root volume in rice (Gai-ping *et al.*, 2006), sorghum (Pritchard *et al.*, 2006), pigeon pea (Saha *et al.*, 2011) and sour orange (Prior *et al.*, 2012). Nie *et al.* (2013) reported an overall increase in root length, diameter and coarse root biomass as well as root: shoot ratio, suggesting plants expand rooting system under elevated CO₂ which would potentially increase long-term below-ground C sequestration. Root turnover rates under elevated CO₂ in several plant species also varied. It decreased (Iversen *et al.*, 2008), increased (Tingey *et al.*, 2000; Norby and Zak, 2011) or remained unchanged (Arnone *et al.*, 2000). Arndal *et al.* (2013) suggested that higher net root production under elevated CO₂ might be a strategy for the plants to cope with increased nutrient demand on whole plant basis.

Atmospheric CO₂ enrichment could alter plant traits that influence soil aggregation, but the response of these traits was species-specific (Cotrufo and Gorrison, 1997). CO₂ enrichment had the potential to increase and stabilize soil aggregation due to increase in root growth (Rogers *et al.*, 1998; Saha *et al.*, 2011). On the other hand, byproducts of microbial activity can form, with the surrounding mineral particles, an organo-mineral sheath around the cells (Chenu, 1993) leading to an increase in macroaggregates (Rillig *et al.*, 1999). Although elevated CO₂ had no

effect on water stable aggregates, it influenced soil moisture, labile C, above-ground and root biomass (Eviner and Chapin, 2002). Total soil C did not change after 9-years CO₂ enrichment (Bazot *et al.*, 2006), but proportion of macroaggregates increased for *Lolium perenne* and not changed for *Trifolium repens* in *Eutric Cambisol* (Six *et al.*, 2001). Dorodnikov *et al.* (2009) also reported no change in aggregate size distribution for *Gleyic Cambisol* after 5-year of elevated CO₂ enrichment exposure.

So far, limited emphasis has been given on the long-term impacts of CO₂ fertilization on rhizosphere soils including growth and activities of plant roots. Leguminous crops offer a unique situation where its typical N-fixing properties interact with soil nutrient dynamics and enzyme activities in ways which are different from non-legumes. Understanding the possible effect of elevated atmospheric CO₂ on morphological changes in roots and nodules along with biological response of rhizosphere soils will have relevance to adaptation to climate change. Moreover, chickpea being the most coveted legume and potential dry land crop, impact assessment of atmospheric CO₂ changes on its growth and development must be documented for continuous augmentation of its production under changed climatic situation.

7.2 Materials and methods

7.2.1 Climate and soils

An open-top chambers (OTC) experiment on chickpea since 2007 has been adopted for the study. The experiment is located at the research farm of the Indian Agricultural Research Institute, New Delhi (28°35' N latitude, 77°12' E longitude, 228.16 m altitude above mean sea level). Soil samples were collected and plant observations were taken during *rabi* (winter) season (mid November-mid April) in 2010-11 and 2011-12 years.

The site has a typical semi-arid climate with dry hot summer and mild winters. The average annual rainfall is 710 mm, of which about 80% is received during crop growing period. Mean daily maximum and minimum air temperature during the experimental years ranged between 10-29 °C and 2-12 °C. The soil belongs to *Typic Haplustept* of Upper Indo-Gangetic Plains with sandy loam in texture, non-calcareous and mild alkaline in reaction. Some major physico-chemical properties of 0-30 cm soil are presented in Table 7.1.

7.2.2 Crop management

A detailed description of the OTCs can be obtained from Saha *et al.* (2011). In two OTCs, CO₂ was maintained at 580±20 µmol mol⁻¹ since crop emergence and the other two chambers were used as controls (no external supply of CO₂). Air temperatures in elevated and ambient CO₂ chambers were nearly identical, but 2-3.8 °C higher than outside. No significant difference in root zone soil temperature (measured by temperature probe) was observed. Healthy and disinfected chickpea (*Cicer arittinum* L.; cv. Pusa 1105- *kabuli* type) seeds were sown manually at 10 cm depth with 30 cm row-to-row and 15 cm plant-to-plant spacing. Basal dose of N: P: K at 20:40:60 was incorporated during seed bed preparation and the sowing was completed within 2nd week of November. After 3 weeks, plants were thinned to a density of 20 plant/ m² in each chamber. Weeding was done manually, while fungicide mixture (Bavistin + Dithane M-45) was sprayed at 50 days after sowing (DAS) to prevent the fungal infection.

7.2.3 Soil analysis

The surface layer (0-15 cm) of the soil was sampled for bulk density, water retention, aggregation, pH, EC, total N, available P and K, soil enzymes and C pools. Nearly 1000 g of bulk soils were collected in triplicate from each chamber. Field-moist samples were thoroughly mixed, passed through 8.0 mm sieve by breaking up the soil along natural plane of weakness, and stored at 4°C in plastic bags till the analysis. Two sets of undisturbed cores (3 from each OTCs) were used to determine soil bulk density (BD) (Veihmeyer and Hendrickson, 1948; Blake and Hartge, 1986) and moisture retention at 0 kPa (hanging water column) and at -33 and -1500 kPa (pressure plate apparatus, Soil Moisture Equipment Corporation, USA) (Klute, 1986). Soil pH and electrical conductivity were measured in a 1:2.5 (w/v) aqueous extract. Total nitrogen content was determined by the micro Kjeldhal method (Bremner, 1960) and available P, extracted with sodium bicarbonate, was determined by acid method (Watanabe and Olsen 1965). Ammonium acetate (pH 7.0) extractable K was determined by flame photometry (Jackson, 1973).

Aggregate stability was measured by Le Bissonais method (Le Bissonais, 1996). This method combines three disruptive tests (pre-treatment) corresponding to different wetting conditions and energies: (a) fast wetting (FW), (b) slow wetting

(SW), and (c) mechanical breakdown (MB). To obtain uniform dryness, aggregate mass was oven-dried overnight at 40°C prior to the analysis. In fast wetting test, approximately 5 g of aggregates was gently immersed in 50 ml of deionized water for 10 min, while in slow wetting test, aggregates was capillary-wetted at -0.3 kPa suction on a tension table for 30 min, and then gently immersed in water (50 ml). In mechanical breakdown test, similar amounts of aggregates were immersed in methanol for 10 min, then transferred into a volumetric flask with 200 ml water, and agitated end-to-end 20 times and left for settling. After these pre-treatments, the samples were transferred into a 0.053 mm sieve previously immersed in methanol. The sieve was moved gently and aggregates on the sieve were collected, oven-dried at 105 °C and dry-sieved using a column of 6 sieves: 2, 1, 0.5, 0.2, 0.1 and 0.053 mm. Four replicates were performed for each pre-treatment. For each of these methods, Aggregate mass on each sieve was recorded and aggregate stability was expressed as mean weight diameter.

7.2.4 Analysis of soil C pools

Total organic carbon (TOC) was determined by using (a) Elementa TOC analyzer (VarioTOC cube) and Schollenberger chromic acid oxidation method (Schollenberger, 1927). The oxidizable organic carbon content and its fractions were estimated following Chan *et al.* (2001). Water-soluble (Ghani *et al.*, 2003) and total acid-hydrolysable (Brink *et al.*, 1960) carbohydrates were determined. Microbial biomass C (MBC) was estimated with 20 g oven-dry equivalent of fresh soil sample by fumigation with ethanol free CHCl_3 and extraction with 0.5 M K_2SO_4 . Organic C in the soil extractant was determined by oxidation with $\text{K}_2\text{Cr}_2\text{O}_7$ (Vance *et al.*, 1987). All the measurements were carried out in 3 replicates.

7.2.5 Soil microbial activity assessment

Soil enzyme assay was carried out at earliest with fresh field moist rhizosphere soils. Prior to analysis, samples were air-dried to equilibrate at room temperature (~25 °C) in the dark for 7 days to attain a constant weight and passed through a 2 mm sieve to obtain a homogeneous soil sample material. Dehydrogenase activity was determined according to Casida *et al.* (1964). Acid and alkaline phosphatase activities were determined by the method as described by Tabatabai and Bremner, (1969) using p-

nitrophenyl phosphate as substrate. β -glucosidase was determined by the method of Eivazi and Tabatabai, (1988) using p-nitrophenyl- β -D-glucopyranoside as substrate. FDA hydrolysis assay was carried out using potassium phosphate buffer (pH 7.6) (Green *et al.*, 2006).

7.2.6 Monitoring of soil carbon dioxide flux

Instantaneous CO₂ flux from the soil inside the crop canopy was measured using Infra-red based continuous flux analyser (LI-8100) system. Carbon dioxide was sampled and analysed with a 20 cm short-term survey chamber in 5 replicates for each OTC.

7.2.7 Root sampling and analysis

Root samples in 4 replicates from 0-15 and 15-30 cm layers in each OTC were collected at peak vegetative (50/48 DAS), 50% flowering (94/72 DAS) and active pod filling (125/90 DAS) stages. Roots were cleaned and washed, and the soil materials adhering to the roots were collected for subsequent analysis. Root samples were soaked overnight in diluted sodium hexametaphosphate solution, floating roots was collected on a 35 mesh sieve and stored at 4°C. Roots were sorted and air-dried thoroughly before placing under a root scanner (LA1600). Scanning was done by using winRHIZO software (REGENT INSTRUMENTS Inc., Canada). Samples were oven-dried at 65°C for 48 hours and weighed. All the parameters were expressed per unit volume of soil core.

7.2.8 Nodule activity Assessment

For nodulation study, separate chickpea plants were selected grown in isolated polythene lining (2 m×1 m) within the OTC plant population. Nodules were harvested at 45 DAS, considering the most active phase of N-fixation for chickpea nodules (Mann *et al.*, 2002). Selected plants were uprooted carefully from moist soil with minimum possible damage to the central root system, and washed thoroughly. The root system was separated for determination of nitrogenase activity. Fresh nodules were detached, weighed precisely (0.0001 g readability) and scanned using win RHIZO system for determination of all physical characters. Parts of the active matured nodule were then separated, weighed, masticated repeatedly in 80% ethyl

alcohol for 15 min and centrifuged at 10000 rpm for 5 min. The supernatant was stored at 4°C and quickly analyzed of leghaemoglobin, proline and phenol contents. Some portions of nodules collected separately were dried at 65°C and then powdered for carbohydrate content and elemental analysis of C and N. All of the above experimentation was done in 4 replicates from each chamber in both the years of experimentation.

The nitrogenase activity was determined by the acetylene reduction assay (Hardy *et al.*, 1968). Briefly here, nodulated roots were cut and shaken slowly to remove the adhering soil particles. Samples were assayed in 30-ml glass tubes sealed with a subseal which was pierced by hypodermic needle, and 10% (v/v) of the air was withdrawn from the sample container and replaced by an equal volume of acetylene gas. After 1 h of incubation at room temperature, 0.5 ml of gas sample was injected into a gas chromatograph (Nucon Series 5250, New Delhi, India), equipped with a flame ionization detector to quantify the ethylene produced. The result was expressed in terms of nanomoles of ethylene formed g^{-1} of nodule fresh mass h^{-1} .

The leghaemoglobin content in fresh nodules was quantified as described by Appleby and Bergersen (1980). It was extracted with sodium phosphate buffer (pH 7.4) and divided equally into two test tubes, each one added with same volume of alkaline pyridine reagent. The hemochrome formed was read at 539 and 556 nm on a spectrophotometer after adding a few crystals of potassium hexacyanoferrate and sodium dithionite, respectively.

Proline content was determined (Bates *et al.*, 1973). Dried plant tissues, roots or leaves (100 mg), were digested in 3 ml of sulfosalicylic acid (3%, w/v) for 30 min at 100°C. The contents were centrifuged at 2000 g for 5 min at 25°C. To 0.2 ml of the supernatant, 0.4 ml of the distilled water and 2 ml of the reagent mixture (consisting of 30 ml glacial acetic acid, 20 ml distilled water and 0.5 g Ninhydrin) were added, and the contents were incubated for 1 h over a boiling water bath. After 1 h, the mixture was cooled and extracted with 6 ml of toluene. The absorbance of the toluene phase was read at 520 nm using toluene as a blank, and the proline content was determined from the standard curve of proline as mg g^{-1} plant tissue.

Phenol content of the nodules was carried out with the Folin-Ciocalteu reagent (Mallick and Singh, 1980) and expressed as mg phenols/100g material in terms of catechol used as standard. The carbohydrate was extracted following the

method of Yih and Clark (1965). Dried powder of nodules was transferred to a glass centrifuge tube, containing 1.5 N H₂SO₄. The sample was centrifuged at 4000 rpm, for 10 min. Water-soluble carbohydrate (WSC) was determined by the anthrone method (van Herwaarden *et al.*, 1998) and for total carbohydrate analysis of nodules, 100 mg of the ground nodule sample was weighed in 6 replicate into boiling tubes. The hydrolyzation was done by keeping the tubes in boiling water bath for 3 hours with 5 ml of 2.5 N-HCl and cools to room temperature and was neutralized with solid sodium carbonate until the effervescence ceases. The volume was adjusted to 100 ml and 1 ml aliquot was taken out after centrifugation for total carbohydrate analysis by the anthrone method described above (Hedge and Hofreiter, 1962). Nodule starch was analyzed as the procedure described by Sadasivam and Manickam (2008) and total C was determined by Elementa TOC analyzer and total N content of dried and powdered nodule samples was determined by Kjeldhal method (Bremner, 1960).

7.2.9 Yield and yield components

After harvest, leaves and stem parts were separated from the pods and oven dried at 80°C to constant weight and the dry weights were recorded. For grain yield, pods were sun-dried and yield parameters were recorded on per plant basis.

7.2.10 Statistical analysis

All the data were statistically analyzed using the SPSS statistical software version 16.0 (SPSS Inc., Chicago) and the significance of differences between means were determined by analysis of variance (ANOVA) procedure (Zar, 2010).

7.3 Results

7.3.1 Hydro-physical properties of rhizosphere soil

Soil hydro-physical properties (Table 7.1) showed a very small but consistent change for elevated CO₂ exposure. Soil bulk density and water retention characteristics showed no significant difference between elevated CO₂ and ambient CO₂ treatments. Whereas, MWD data analysis showed 37.8% (p=0.022) increase for mechanical break down and 36.3% (p=0.005) increase in fast wetting process, but no significant difference for slow wetting process under elevated CO₂ treatment.

7.3.2 Carbon and associated nutrient pools

Although a non-significant increasing trend was observed for total organic C and no significant change in total soil N, we observed a non-significant widening of soil C:N ratio for elevated CO₂ treatment (Table. 7.2). While, available K showed no significant influence of CO₂ enrichment effect, available P showed a sharp tendency of increase of nearly 42.7% ($p < 0.05$) and 29.3% ($p < 0.05$) due to CO₂ fertilization effect, during 2010-11 and 2011-12, respectively.

Different pools of rhizosphere C were affected (Fig. 7.1) in various degrees, but only carbohydrate C and microbial biomass C showed significant variations. Acid hydrolysable carbohydrate fraction decreased in both elevated and ambient CO₂ exposure, but the magnitude was 27% ($p < 0.01$) and 14.1% ($p < 0.01$) higher under elevated CO₂ exposure as compare to ambient in 2011-12 and 2011-12, respectively. Although the water soluble fraction of carbohydrate C reduced by 12.7% and 40.5% under elevated CO₂, but as compared to increasing magnitude of average 19% under ambient CO₂ condition during two consecutive years of our experimentation. Although the trend was not found to be statistically significant but Scholenberger C showed an average decrease of 20% under elevated CO₂, as compared to an increase of 11% under ambient condition. The most labile oxidizable C (12N) showed a significant trend of increase by 10.2% under elevated CO₂, while we observe a non-significant declining trend for other stable soil C pool under elevated CO₂ treatment in varying magnitude. We observed an average non-significant decreasing trend by 15%, 16.6% and 19.5% for 36N, 24N and 18N fractions under elevated CO₂ treatment, while those fractions were found to get amplified by 19%, 15.8% and 23.1%, respectively, under ambient CO₂ condition. Microbial biomass C in elevated CO₂-exposed soil showed a significant increase of 48.4% ($p < 0.01$) over ambient CO₂ treatment. Soil CO₂ flux was 1.3 times higher under elevated CO₂ indicating an increased microbial respiration. Although temporal variation of CO₂ concentration in soil atmosphere ranged widely (0.96 to 13.2%), there was an average increase of 27.5% under elevated CO₂ condition (Fig. 7.2).

7.3.3 Enzyme activity

All the enzymes activities were significantly higher under elevated CO₂. β glucosidase activity was higher by 18.0% ($p < 0.01$) and 21.25% ($p < 0.01$) in two years, under

elevated CO₂ treatment and so was the alkaline phosphatase activity amplification by 69.0% ($p < 0.01$) and 37.5% ($p < 0.01$) during 2010-11 and 2011-12, respectively (Fig. 7.3). Increase in acid phosphatase activity was varied widely by 67% ($p < 0.01$) in 2010-11 and 12% ($p < 0.05$) in 2011-12 for elevated CO₂ treatment. On average, 47% ($p < 0.01$) increase in soil dehydrogenase activity was recorded during both of the years of our experimentation, while the increase in FDA hydrolysis activity was varying between 64.6% ($p < 0.01$) and 93.6% ($p < 0.01$) under elevated CO₂ during 2010-11 and 2011-12, respectively.

7.3.4 Root characters

Root weight, length and surface area densities were higher in 0-15 cm layer (Table 7.3). Root dry weight density showed a significant increasing trend under elevated CO₂ fertilization. As compared to 40.1% ($p < 0.01$) and 4%, increase at vegetative crop growth stage during two consecutive years of experimentation, root dry matter density increased by 59% ($p < 0.01$) and 38% ($p < 0.01$) at 50% flowering stage under elevated CO₂ treatment, during 2010-11 and 2011-12, respectively. The maximum accumulation was observed at active pod filling stage, where it showed an increase of 250% ($p < 0.01$) and 127.4% ($p < 0.01$) due to atmospheric CO₂ fertilization effect. Root length density also showed a similar trend as it increased by 21.5 % ($p < 0.05$) and 16.5% ($p < 0.05$) at crop vegetative stage during 2010-11 and 2011-12, respectively. It was followed by 36.6% ($p < 0.01$) and 72.3 % ($p < 0.01$) increase in root length density at 50% flowering and active pod filling during 2010-11 respectively. In the next year, it increased by 51.3% ($p < 0.01$) at 50% flowering and 25% ($p < 0.05$) at pod maturity stage. An average increase of 30% ($p < 0.05$) root surface area density was observed at vegetative stage, during both the years of experimentation, which was amplified to 36.8% ($p < 0.01$) and 74% ($p < 0.01$) at 50% flowering during two consecutive years. At active pod filling stage the change in magnitude of root surface area density was non-significant though it an increased by 24.7% and 13.2%, during 2010-11 and 2011-12, respectively. Root volume density increased on an average by 49% ($p < 0.01$) and 37.3% ($p < 0.05$) at all the crop growth stages, during 2010-11 and 2011-12, respectively. Although average root diameter showed an increase of 11.7% and 15.5% during 2010-11 and 2011-12, respectively, but the increase was non-significant statistically.

At 15-30 cm depth similar elevated CO₂ effect was move on. During 2010-11, a significant increase in root dry matter density was found under elevated CO₂ and that was varying from 28.4% ($p < 0.05$) at vegetative growth stage, 58% ($p < 0.01$) at 50% flowering and 69.3% ($p < 0.01$) at active pod filling stage. The root length density and surface area density, maintained a continuously increasing trend up to active pod filling stage with about 50% ($p < 0.01$) higher root length density along with 60 % ($p < 0.01$) increase in surface area density for elevated CO₂ treatment than that of ambient CO₂, over all the crop growth stages. The root volume density increased 60% ($p < 0.05$) at vegetative stage, which was got amplified to 75.2% ($p < 0.01$) at 50% flowering and 81.5% ($p < 0.05$) at active pod filling stage. The average root diameter was found to get increased by about 25.4% ($p < 0.05$) for elevated CO₂ treatment all over the crop growth period. During 2011-12, no effect of elevated CO₂ fertilization was observed at 15-30 cm depth, except for root surface area density, which showed an increase of 25% ($p < 0.05$) and 19.6% ($p < 0.05$) at 50% flowering and active pod filling stage, respectively.

7.3.5 Nodule physical characteristics

Nodulation being the most important character of the leguminous chickpea was highly affected due to elevated CO₂ exposure (Table 7.4). The total number of nodules appeared on its central and lateral root system were increased by 38.8 % ($p < 0.01$) and 37.5 % ($p < 0.05$) at vegetative stage during 2010-11 and 2011-12, respectively. The increasing trend continued at 50% flowering stage by 48.2% ($p < 0.05$) and 63.1% ($p < 0.01$) during two respective years of our experimentation. At active pod filling stage, the number of nodules were higher by 36.1% ($p < 0.05$) and 58.8% ($p < 0.05$) under elevated CO₂, during both of the years of experimentation.

Total nodule dry biomass showed an increasing trend under elevated CO₂ (Table 7.4). The trend was very prominent at 50% flowering and active pod filling stage as it increased by 73.1% ($p < 0.01$) and 32.5% ($p < 0.01$) respectively, during 2010-11. In next year the magnitude of increase was 93% ($p < 0.05$) at 50% flowering and 43.7% ($p < 0.05$) at active pod filling stage. Plants grown under elevated CO₂ were bearing nodules with lower relative water content by 9.4% ($p < 0.05$) and 7.2% ($p < 0.05$) at vegetative crop growth stage during the two consecutive years. The difference reduced at later crop growth stages. A significant reduction in nodule

specific volume by 29.5% ($p < 0.05$) and 39.1% ($p < 0.05$) was observed at vegetative crop growth stages during 2010-11 and 2011-12, respectively. The reduction was most prominent at 50% flowering stage by 37.2% ($p < 0.01$) and 71.8% ($p < 0.01$), during 2010-11 and 2011-12, respectively. At active pod filling stage, it was about 55.7% ($p < 0.01$) and 22.8% ($p < 0.05$) higher under elevated CO_2 treatment, during both the years of experimentation. Nodule specific surface area increased by 58.3% ($p < 0.01$) and 25.6% ($p < 0.01$) at vegetative stage, under elevated CO_2 treatment during 2010-11 and 2011-12, respectively. At active pod filling stage similar trend was found with magnitude of 24.2% ($p < 0.05$) during 2010-11 and 20.4% ($p < 0.05$) during 2011-12.

7.3.6 Nodule Biochemical characters

Averaging the values for two years experimentation, we observed no significant change in net C:N ratio of nodule, although total C contents in nodules increased by 14.8% ($p < 0.05$) and 19% ($p < 0.01$) with a concomitant rise in total nodule N by 9.3% ($p < 0.05$) and 12.1% ($p < 0.05$) under elevated CO_2 exposure, during 2010-11 and 2011-12 respectively (Fig. 7.4a). Nodule carbohydrate content also showed significant increase by 14.1% ($p < 0.01$) and 27.1% ($p < 0.01$) under elevated CO_2 , during 2010-11 and 2011-12 respectively. Soluble sugar concentration increased by 20.7% ($p < 0.01$) and 28.4% ($p < 0.01$) during the two consecutive years of experimentation with concomitant average increase in stable starch fractions by 31% ($p < 0.01$) over both of the years. Nitrogenase activity assessed by acetylene reduction activity of nodules, increased by 38% ($p < 0.05$) during 2010-11 and 92.1% ($p < 0.01$) during 2011-12. A significant increase in leghaemoglobin content by 58.1% ($p \leq 0.05$) and 74% ($p \leq 0.01$) was observed for elevated CO_2 treatment (Fig. 7.4b). Although the phenol content in the chickpea nodules was insensitive to the atmospheric CO_2 enrichment but an average increase in proline content in nodules was observed by 19.4% ($p \leq 0.01$) and 29.7% ($p \leq 0.01$) with an of average 6% ($p \leq 0.05$) reduction in relative water content for nodules grown under elevated CO_2 during 2010-11 and 2011-12, respectively.

7.3.7 Yield components

The total number of pods per plant, total pod weight per plant and seed weight per plant also increased significantly ($p \leq 0.01$) under elevated CO₂ (Fig. 7.5). At harvest, number of matured pods plant⁻¹ were significantly higher by 108% and 53%, with concomitant increase in seed yield by 118% and 45%, during 2010-11 and 2011-12, respectively. The seed yield (weight of seed per plant) increased significantly by 107.7% ($p \leq 0.01$) and 43% ($p \leq 0.01$) during two consecutive years of experiment, as the number of pods per plant increased significantly. The harvest Index (HI) increased significantly by 26.5% ($p \leq 0.01$) and 13.4% ($p \leq 0.05$) under elevated CO₂ in 2010-11 and 2011-12, respectively.

7.4 Discussion

Elevated CO₂ brings significant increase in root morphological characters, mostly at 50% flowering and active pod-filling stages. The magnitude of changes are larger in 0-15 cm layer, however variations between years were also evident (Table 7.3). Root length and surface area densities typically increased, as compared to other root parameters. Results suggest an increased dry matter allocation to roots of chickpea, which is supported by a number of previous studies (van Ginkel *et al.*, 2000; Gai-ping *et al.*, 2006; Austin *et al.*, 2009; Bhattacharyya *et al.*, 2013). No effect of elevated CO₂ on root biomass is also reported (Pritchard *et al.*, 2001; Haase *et al.*, 2007; Brown *et al.*, 2007). Improved root growth has been associated with increase in microbial activities in clover (Schortemeyer *et al.*, 1997). We also observed positive response in soil microbial biomass C (1.6 to 2.7 times higher under elevated CO₂). Increase in microbial biomass C is also reported by Li *et al.* (2004c), Das *et al.* (2011) and Bhattacharyya *et al.* (2013).

There is no general consensus on the change in soil C through CO₂ fertilization, although increase in soil organic C in diverse temperate ecosystems has been reported (Jastrow *et al.*, 2005; Iversen *et al.*, 2012). Total C and N increased marginally (along with marginal increase in C:N ratio) although both of the changes are non-significant. Trends in widening C:N ratio indicates more biologically active rhizosphere, as argued by Anderson *et al.* (2011). Except labile C (12 N modified W-B and MBC), all the pools of C including Scholtenberger C had shown decline under elevated CO₂ (Fig. 7.1). Increased microbial response under atmospheric CO₂

fertilization is supported by other studies (De Graaff *et al.*, 2006, Carney *et al.*, 2007; Inubushi *et al.*, 2011; Saha *et al.*, 2011; Das *et al.*, 2011; Anderson *et al.*, 2011 ; Bhattacharyya *et al.*, 2013). This suggests that greater root growth results in augmented C inputs (Lukac *et al.*, 2003; Godbold *et al.*, 2006) and thus increasing the active labile C fraction in soil (Hoosbeek *et al.*, 2006; Kant *et al.*, 2007; Ross *et al.*, 2013). This further implies dominating impact of CO₂ on soil microbial activities and their subsequent controls over ecosystem N dynamics (Hu *et al.*, 2006), triggered through CO₂ stimulation of symbiotic N₂ fixation (Rogers *et al.*, 2006). Total carbohydrate C declined from their initial values, the decrease are further in elevated CO₂. This explains higher decomposition of organic substrate by microbes under elevated CO₂. However, while water soluble carbohydrate C declined under elevated CO₂, ambient CO₂ resulted in a net increase. This apparent contrasting behavior suggests still higher activity of microbes in utilizing the residual (labile) water soluble carbohydrate C under the elevated CO₂ condition. A tracer study indicated that significant quantities of plant C and N are translocated to roots and then into the rhizosphere soil in higher quantities under elevated CO₂ (Yasmin *et al.*, 2010). Increased allocation of C to root nodules (both total and carbohydrate C), of chickpea induce the crop to fix more N, as evidenced by greater nodule N (Rogers *et al.*, 2006), and thereby stabilizing the C:N ratio.

In an earlier OTC study with pigeon pea, Saha *et al.* (2011) reported increase in both labile and recalcitrant C pools indicating a gross soil C accumulation over years. However in the present study with chickpea, decreasing trend of recalcitrant fractions of C is observed indicating decline in soil C storage (Carney *et al.*, 2007) with subsequent increase in labile fraction. Plants increase soil C through rhizodeposition, while loss in soil C is mostly governed by microbial mineralization. Pigeon pea has a bigger canopy and root architecture compared to that of chickpea and also more efficient in N-fixation (Bidlack *et al.*, 2001). This facilitate in higher C rhizodeposition (Wichern *et al.*, 2008) under pigeon pea crop. Less C rhizodeposition may trigger microbes to utilize native recalcitrant C pool in a greater way. Decreasing recalcitrant C in rhizosphere in our study might be indicating the dominance of microbial activity over rhizodeposition process in chickpea.

Our results of increased soil CO₂ flux and concentration (Fig. 7.2) are in agreement with Moore *et al.* (2008), but in contrast to no change in soil and root

respiration (Fitter *et al.*, 1996). Significant positive relations between root biomass and root respiration were reported (Pregitzer *et al.*, 2000). The increase of microbial respiration is closely related to the enhancement of the amount of root exudation and above-ground litter (Zak *et al.*, 2000). The greater microbial respiration in our study corroborates with greater root biomass, higher litter inputs in elevated- compared to ambient-CO₂ chambers (Bernhardt *et al.*, 2006; George *et al.*, 2003). Higher atmospheric CO₂ concentration promoted soil microbial respiration, which resulted in more labile C fractions in soil (Lagomarsino *et al.*, 2006; Bhattacharyya *et al.*, 2013; Ross *et al.*, 2013). This favored microbial immobilization process (Lagomarsino *et al.*, 2008) with enhanced associated soil enzyme activity (discussion follows). Overall, the rhizosphere under chickpea becomes biologically more active under CO₂ enrichment, which is supported by increased flux and concentration of CO₂ in soil as observed in this study.

Elevated CO₂ influences soil enzyme activities through altering the quantity and quality of plant-derived substrates entering the soil (Zak *et al.*, 2000; Li *et al.*, 2010) (Fig. 7.3). β -glucosidase, an extracellular C-transforming enzyme, acts as a potential indicator of biological soil quality and is actively involved in degradation of soil organic matter specially the cellulose (Turner *et al.*, 2002). Increased activity of β glucosidase in CO₂ chambers is in agreement with other studies (Das *et al.*, 2011, Kim and Kang, 2011, Bhattacharyya *et al.*, 2013), implying higher rates of soil organic matter degradation under elevated CO₂. We found a significant increase in acid and alkaline phosphatase activities under elevated CO₂ as also reported by others (Lagomarsino *et al.*, 2008; Das *et al.*, 2011; Manna *et al.*, 2013). Alkaline phosphatase is derived from microorganisms only, while acid phosphatases are contributed by both root with its associated mycorrhiza and other fungi (Tarafdar and Marschner, 1994). Evidence is also available that plant roots produced phosphatase enzyme capable of dephosphorylating the organic P (Greaves and Weblay, 1965). Our results suggested that alkaline phosphatase activity was more than the acid phosphatase, as the soil used in this study was slightly alkaline in nature (Das *et al.*, 2011). However, the increase over ambient CO₂ was 13.8% higher for acid phosphatase due to better substrate availability for microbial activity (Tabatabai and Bremner, 1969) with about 36% increase of available P in soil solution at harvest under elevated CO₂ treatment (Table. 7.3). Chickpea is reported to exude

progressively higher amounts of organic acids in soil and increases the availability of phosphorus to the plant (Veneklaas *et al.*, 2003). Our soil was marginally deficient in available P (9.62 mg kg^{-1} ; Muhr *et al.*, 1965), and elevated CO_2 brought amplification of processes like rhizosphere acidification, exudation of organic anions and secretion of phosphatases from plant roots in response to P deficiency (Hinsinger *et al.*, 2005; Richardson *et al.*, 2005). This elucidates positive impact of CO_2 fertilization on mobilizing P from soil P reserve (insoluble), especially in P-deficient soils. Increase in soil dehydrogenase and FDA activities under elevated CO_2 are supported by a number of other studies (Das *et al.*, 2011; Bhattacharyya *et al.*, 2013); however no change was also reported in rice (Manna *et al.*, 2013). Higher atmospheric CO_2 increases the allocation of photosynthates to roots with increased root growth and rhizodeposition (Haase *et al.*, 2007; Anderson *et al.*, 2011) and microbial respiration (De Graaff *et al.*, 2006). Increase in enzyme activities promote better nutrient availability in soil (Jin and Evans, 2007) and also increase in number and activities of microbes (Dhillon *et al.*, 1996). Since microbes use the translocated photosynthates as C and energy source (leading to higher activities and build up of microbial biomass), increase in labile soil C under elevated CO_2 validates the hypothesis of higher exudation of photosynthates from the roots leading to proliferation of microbes. Larger enhancement of microbial activities might reduce soil C stock by bringing recalcitrant pools to labile fractions, which is lost as CO_2 flux. This may act as the counterbalance to increased soil C addition (litter and rhizodeposition) through atmospheric CO_2 enhancement. Therefore, higher C sequestration in CO_2 enriched environment as widely envisaged, might not be a possibility. Elevated CO_2 will constrain the potential for net gains in soil C suggesting a limited capacity of earth's ecosystems to stabilize atmospheric CO_2 and slow global warming (Carney *et al.*, 2007). Role as sink for long-term C storage under increased atmospheric CO_2 has also been questioned in forest soils (Schlesinger and Lichter, 2001).

Elevated CO_2 boosts nodulation in chickpea as also reported by Schortemeyer *et al.* (2002) and Lee *et al.* (2003). Increase in nodule number and biomass was recorded mostly during post anthesis, although the difference in number between elevated and ambient condition reduced in 2nd year of experiment possibly due to delay in sowing (Table 7.4). However, we could not observe any positional difference in nodule number between main or lateral root branches (data not presented). Dry

weight and specific surface area of individual nodule increased due to atmospheric CO₂ enrichment, whereas significant reductions in specific volume and relative water content were observed. Biomacromolecular composition in nodules was significantly improved under elevated CO₂ (Fig. 7.4a). Increase in total C (Morgan *et al.*, 2005a; Rogers *et al.*, 2006) and higher soluble sugar (Aranjuelo *et al.*, 2009b) indicates greater translocation of these to root nodules. The starch concentration was also enhanced (Cabrerizo *et al.*, 2001) along with nitrogenase activity (Temperton *et al.*, 2003a) and leghaemoglobin content (Haase *et al.*, 2007) in nodules (Fig. 7.4b). We propose a possibility of increased N-fixation and plant N immobilization of chickpea under elevated CO₂ condition (Hungate *et al.*, 1999; Fischinger *et al.*, 2010) as both the above-ground biomass and below-ground nodule mass significantly increased under elevated CO₂ environment. Under elevated CO₂, significant increase in nodule proline concentration by 24% ($p < 0.01$) was observed with 6% reduction in nodule relative water content (Serraj *et al.*, 1999). Being a reliable environmental stress indicator (Claussen, 2005), proline accumulation in nodules signifies possible osmoprotection mechanism from elevated CO₂ induced moisture stress (reduced nodule relative water content) and thus reducing oxidative stress (Arrese-Igor *et al.*, 2011). Ultimately, increased nodule osmolyte concentration due to enhanced translocation of synthesized carbohydrate towards the chickpea nodules, resulting from elevated CO₂ induced increase in shoot photosynthesis, (Huang *et al.*, 1975; Serraj *et al.*, 1999) was the major influencing factor for the increased nodule nitrogen fixation, as well as the C sink strength of elevated CO₂ grown chickpea nodules (Aranjuelo *et al.*, 2008).

Substantial increase in seed yields and harvest index (dry matter increased but not as much as seed yield) were recorded under elevated CO₂ (Fig. 7.5). Although delayed sowing in 2nd year caused reduction in HI, percent increase over ambient condition was similar as ~13% (Fig. 7.5). Contradictory results of decrease in HI for rice (Kim *et al.*, 2003), soybean (Morgan *et al.*, 2005b; Sun *et al.*, 2009) and pigeon pea (Saha *et al.*, 2012); increase in HI for wheat (Kimball *et al.*, 1995; Wheeler *et al.*, 1996) and no effect on HI of lupin (Palta and Ludwig, 2000) have been reported.

7.5 Conclusions

Under elevated CO₂ environment, there is an altered number of infection sites and nodule morphology. At the same time, changes in carbohydrate supply to the micro-symbiont, or increased root exudation of signal compounds (malonate, Haase *et al.*, 2007), an essential function in symbiotic N₂ fixation (Ross *et al.*, 2004; Rogers *et al.*, 2006), might occur. As a leguminous crop, chickpea may be able to offset the CO₂-induced enhanced N-requirement in the rhizosphere by increasing N-fixation (Fischinger *et al.*, 2010) and therefore, play a key role in maintaining the C:N balance in the ecosystem (Marilley *et al.*, 1999; Haase *et al.*, 2007). Simultaneously, crop yield and harvest index is also increased as result of CO₂ fertilization effect (Prévost *et al.*, 2010). Briefly, chickpea has the potential for supplying nodule photosynthate requirements for sustaining higher N₂ fixation, while excess C supply is used to promote further nodulation under elevated CO₂ environment. Therefore it may be concluded that chickpea has the ability to regulate symbiotic N fixation according to its N requirement under elevated CO₂ atmosphere providing a strong competition advantage to the plants.

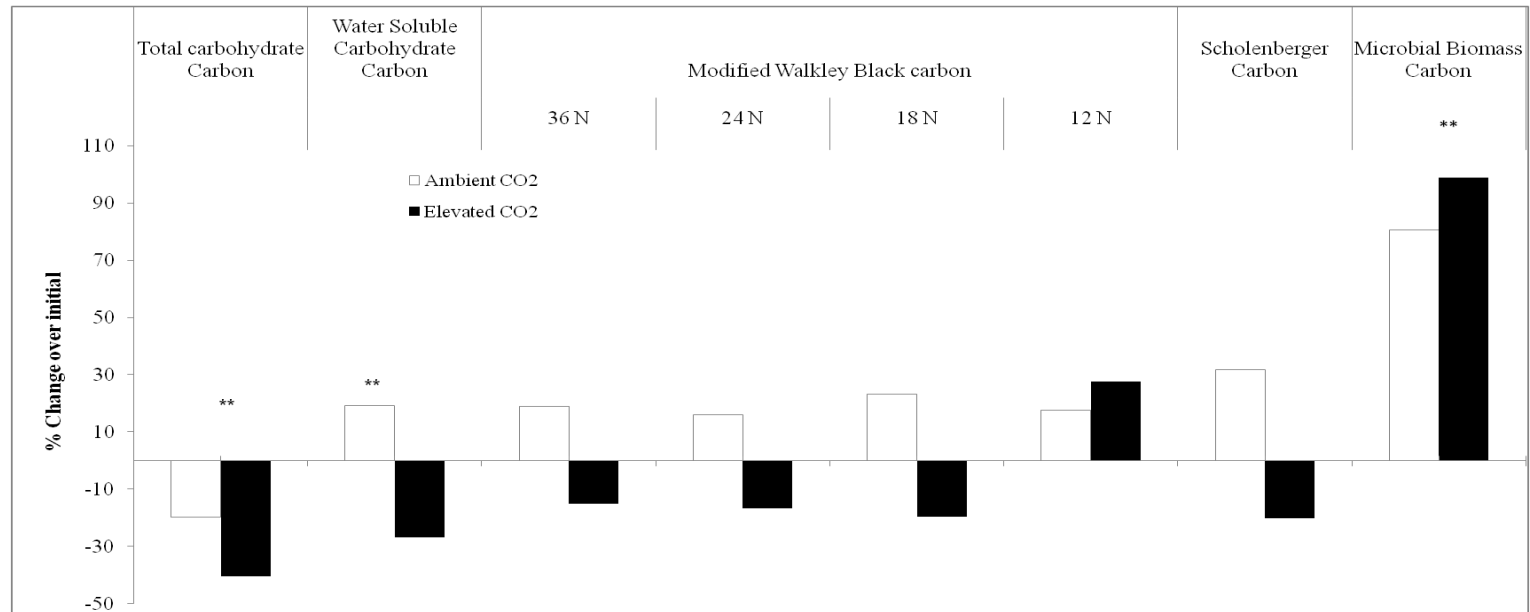


Fig. 7.1: Different fractions of carbon in the rhizosphere soil under elevated CO₂.

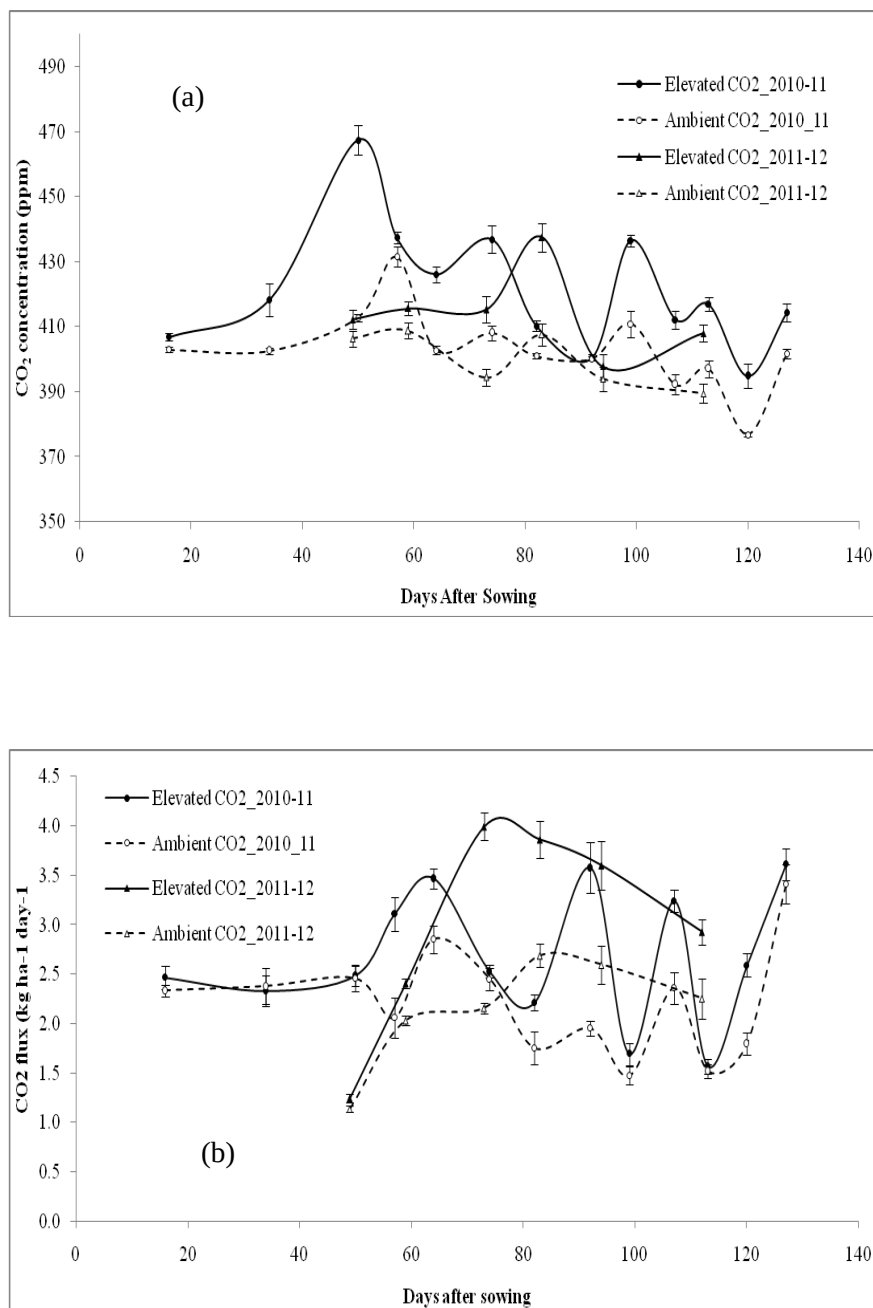


Fig. 7.2: Instantaneous soil CO₂ concentration (a) and flux within the plant canopy (b) under elevated and ambient atmospheric CO₂ levels in 2010-11 and 2011-12.

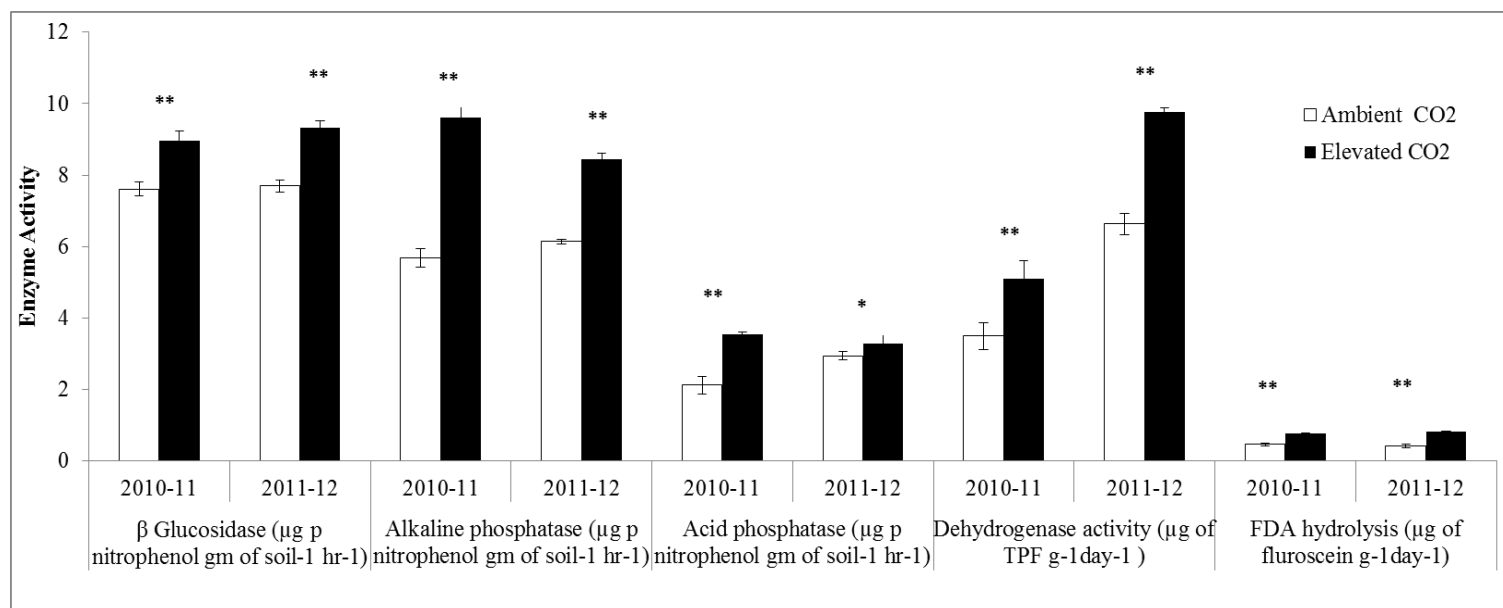


Fig. 7.3: β Glucosidase ($\mu\text{g p nitrophenol gm of soil}^{-1} \text{ hr}^{-1}$), Alkaline phosphatase ($\mu\text{g p nitrophenol gm of soil}^{-1} \text{ hr}^{-1}$), Acid phosphatase ($\mu\text{g p nitrophenol gm of soil}^{-1} \text{ hr}^{-1}$), Dehydrogenase activity ($\mu\text{g of TPF g}^{-1}\text{day}^{-1}$), and FDA hydrolysis ($\mu\text{g of fluroscein g}^{-1}\text{day}^{-1}$) activities in soil as influenced by elevated CO₂ exposure during 2010-11 and 2011-12.

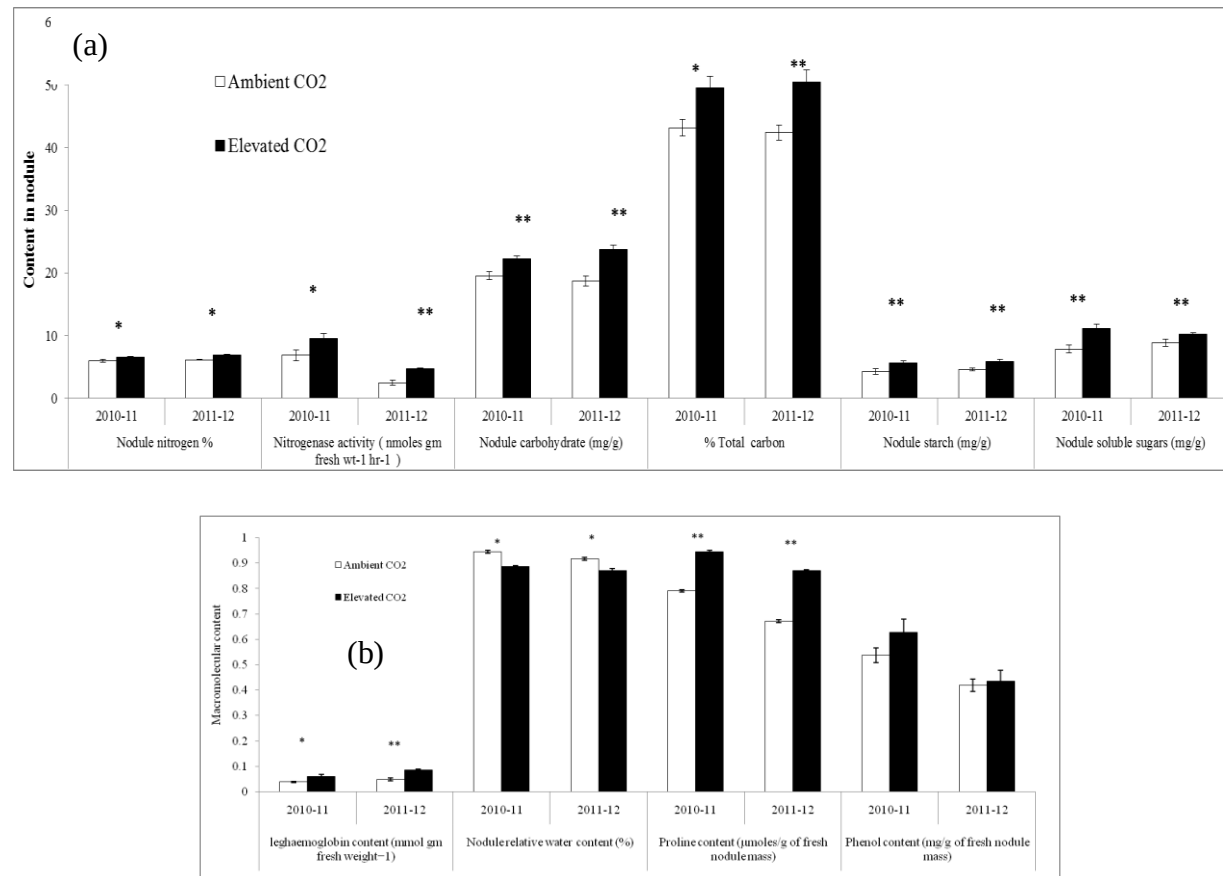


Fig. 7.4: Changes in (a) bio molecular composition and (b) biochemical response of chickpea nodules under elevated and ambient CO₂ during 2010-11 and 2011-12

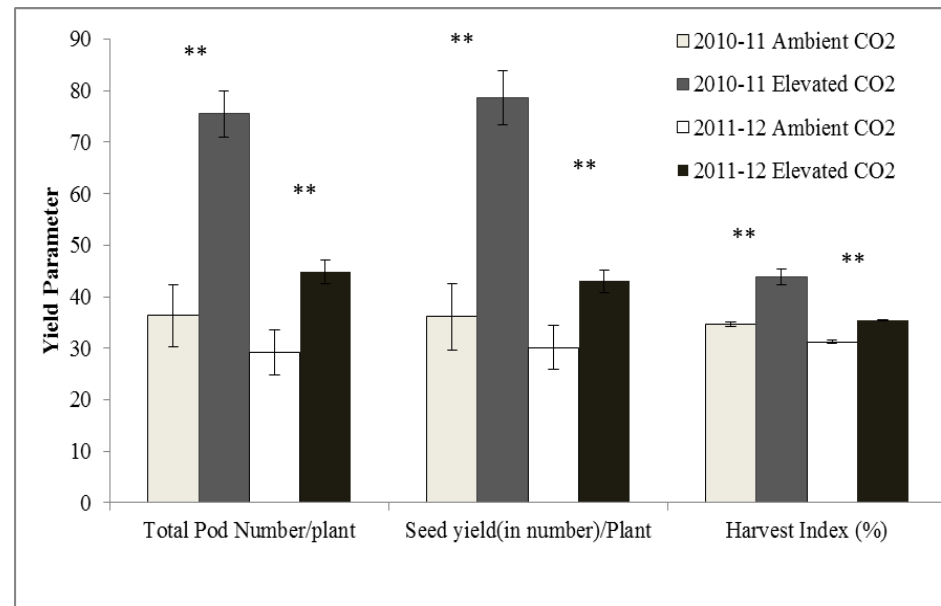


Fig. 7.5: Yield parameters at harvest under elevated and ambient CO₂ in 2010-11 and 2011-12

Table 7.1: Long term CO₂ fertilization effect on soil physical properties

Treatment	Hydraulic conductivity cm h ⁻¹	Bulk density (Mg/m ³)	Mean weight diameter of aggregates (mm) following pre-treatment of			Water retention (m ³ m ⁻³) at			Soil saturation extract	
			Mechanical breakdown	Fast wetting	Slow wetting	0	33	1500	Soil reaction (pH)	Electrical conductivity (dS m ⁻¹)
Ambient CO ₂	1.09	1.51	0.46	0.39	0.46	0.30	0.15	0.08	7.90	0.71
Elevated CO ₂	1.17	1.57	0.63	0.53	0.48	0.32	0.16	0.09	7.84	0.64
p value	0.720	0.134	0.022	0.005	0.681	0.241	0.659	0.801	0.80	0.921

Table 7.2: Soil C, N, P and K changes as mediated by elevated CO₂ (* denotes significant difference at (p<0.05) level for both of the years

	Initial	2010-11		2011-12	
		Elevated CO ₂	Ambient CO ₂	Elevated CO ₂	Ambient CO ₂
Total Organic Carbon (%)	1.45	1.76	1.63	1.61	1.53
Total N (%)	0.16	0.132	0.113	0.119	0.126
C:N ratio	9.06	13.43	12.16	13.56	12.19
Available P (mg/kg)	9.621	14.304*	10.3	13.125*	10.152
Available K (mg/kg)	57.48	46.6	48.97	41.86*	51.91

Table 7.3: Morphological characteristics of chickpea roots in 0-15 and 15-30 cm layers under elevated CO₂ (E-CO₂) and ambient CO₂ (A-CO₂)

Year of experiment	Depth (cm)		Vegetative stage		50% Flowering		Active pod filling	
			E- CO ₂	A-CO ₂	E- CO ₂	A-CO ₂	E- CO ₂	A-CO ₂
2010-11	0-15	Dry matter density (g cm ⁻³ x 10 ⁻⁵)	205**	146	427**	268	498**	142
		Root length density (cm cm ⁻³)	1.93*	1.58	3.18**	2.30	2.26**	1.31
		Root surface area density (cm ² cm ⁻³ x 10 ⁻²)	45.1*	33.9	66.5**	48.6	45.1*	36.1
		Root volume density (cm ³ cm ⁻³ x 10 ⁻⁴)	97**	62	114**	77	69**	42
		Root diameter (cm x 10 ⁻²)	75.4	69.4	70.5	62.6	82.3	72.3
	15-30	Root dry matter density(g cm ⁻³ x 10 ⁻⁵)	95*	74	239**	151	281**	165
		Root length density (cm cm ⁻³)	0.39**	0.25	0.69**	0.47	0.81**	0.54
		Root surface area density (cm ² cm ⁻³ x 10 ⁻²)	13.8**	8.2	14.6*	9.3	23.7**	14.9
		Root volume density(cm ³ cm ⁻³ x 10 ⁻⁴)	13.0*	8.1	14.2**	8.2	22.1*	12.1
		Root diameter (cm x 10 ⁻²)	34.4*	29.2	34.6*	28.4	45.6*	35.13
2011-12	0-15	Root dry matter density(g cm ⁻³ x 10 ⁻⁵)	225	216	674*	488	660**	293
		Root length density (cm cm ⁻³)	0.61*	0.543	1.37**	0.91	1.02*	0.81
		Root surface area density (cm ² cm ⁻³ x 10 ⁻²)	17.5*	13.5	25.2**	14.4	28.3	25.0
		Root volume density (cm ³ cm ⁻³ x 10 ⁻⁴)	28.8*	22.3	42.1*	28.7	53.3*	40.1
		Root diameter (cm x 10 ⁻²)	36.3	31.8	67.1	55.25	51.5	46.4

	15-30	Dry matter density($\text{g cm}^{-3} \times 10^{-5}$)	26	22	124	126	234	25
		Root length density (cm cm^{-3})	0.21	0.19	0.37	0.35	0.15	0.13
		Root surface area density ($\text{cm}^2 \text{cm}^{-3} \times 10^{-2}$)	9.1	7.2	11.0*	8.8	10.6*	8.9
		Root volume density ($\text{cm}^3 \text{cm}^{-3} \times 10^{-4}$)	8.8	6.9	16.2	20.2	18.2	17.2
		Root diameter ($\text{cm} \times 10^{-2}$)	16.6	16.9	28.4	24.3	27.6	29.7

Table 7.4: Physical characteristics of chickpea nodules as influenced by elevated CO₂

Parameters	Crop growth stages	2010-11		2011-12	
		Elevated CO ₂	Ambient CO ₂	Elevated CO ₂	Ambient CO ₂
Total number of nodules/ plant	Vegetative	50**	36	22*	16
	50% Flowering	43*	29	31**	19
	Active pod filling	49*	36	27*	17
Total nodule dry mass (mg) /plant	Vegetative	39.8*	23.1	15.9	11.8
	50% Flowering	151.1**	87.41	56.9*	29.5
	Active pod filling	138.6**	104.4	65.1*	45.3
Relative Water Content (%)	Vegetative	84.3*	92.9	85.2*	91.8
	50% Flowering	76.6*	85.2	89.1	89.4
	Active pod filling	93.3	95.9	90.9	87.7
Specific volume (cm ³ /g fresh wt)	Vegetative	0.44*	0.57	0.92*	1.28
	50% Flowering	0.91**	1.45	1.03**	1.77
	Active pod filling	0.52**	0.81	0.71*	0.92
Specific surface area (cm ² /g fresh wt)	Vegetative	31.82**	20.09	23.48*	18.68
	50% Flowering	26.01	24.33	17.78	16.22
	Active pod filling	13.27*	10.68	7.77*	6.45

{* and ** denotes significant difference at 0.05 and 0.01 level respectively}

8. RESEARCH PAPER 5

Impacts of elevated CO₂ on produce quality of chickpea

Abstract

Experiments on chickpea (*Cicer aurietinum* L.) were performed in open-top-chambers during 2010-12 to assess effects of elevated CO₂ on the quality of the produce. Although no change was observed in physical dimension of seeds, an increase in seed hydration characteristics were recorded under atmospheric CO₂ enrichment. Both the hydration and swelling capacities of seeds were increased by 15.6 and 27.2% in the two experimental years. A close association of swelling index with hydration index and swelling capacity of seeds ($p < 0.05$) was observed. Seed germination reduced by 45-47% with increased seed leachate conductivity by 10-17% under elevated CO₂. Seedling vigour increased, although root and shoot length and dry seedling biomass showed negligible changes. Decrease in protease activity along with reduced seed viability was observed under elevated CO₂ with no change in phosphatase activity. Increase in seed carbon content along with decrease in seed N resulted in widening of C:N ratio, with subsequent dilution of K, Ca, S and Zn by 31.7, 27.1, 30.6 and 38.5%, respectively. No appreciable changes were recorded in pH, bulk density and foaming characteristics of chickpea flour, although water capacity of flour increased with a subsequent reduction in oil absorption capacity (27-44%). Gelation property improved by 4% with reduction in seed protein content and increase in carbohydrate fractions. Atmospheric CO₂ enrichment brought reduction in phenol and crude fibre content in seeds by 24.6% and 34% respectively. Results suggests that elevated CO₂ has negative impact on seed quality (reduced germination characteristics) along with nutritional imbalance caused by increased C content and subsequent dilution of N, K Ca, Zn and S content in seeds. Additionally, reduction in N content, increased water absorption and decreased oil absorption capacity indicates significant deterioration in grain quality through atmospheric elevated CO₂ exposure.

Key words: C:N ratio, seed germination, flour quality, nutrient content

8.1 Introduction

Anthropogenic CO₂ emission of 9.5 ± 0.5 Pg C yr⁻¹ with average global concentration of 391.38 ± 0.13 ppm in the atmosphere (40% increase over pre-industrial level (Le Quéré, 2012; Högy *et al.*, 2009c), is one of the major threats today. Increasing fossil fuel burning and land use changes are most invading marks of human civilization on our planet (Wang *et al.*, 2009). It is expected to vary between 470 and 570 $\mu\text{mol mol}^{-1}$ by 2050, and is increasing at a rate of 1.5-1.8 $\mu\text{mol mol}^{-1} \text{yr}^{-1}$ (IPCC, 2007). Since CO₂ is the major greenhouse gas in the atmosphere, this increase will affect global climate in near future, which in turn, will affect most functioning process of plant–soil interactions system. Increased CO₂ is also likely to cause a significant increase in yields of most C₃ crops, (Ghannoum *et al.*, 2000; Morgan *et al.*, 2001; Jaggard *et al.*, 2010), with increased possibilities of considerable changes, by altering the qualitative (nutritional and biochemical characteristics) and quantitative modifications of the produce for a variety of agricultural crops, like, rice (Yang *et al.*, 2007; Wang *et al.*, 2011), wheat (Blumenthal *et al.*, 1996; Erbs *et al.*, 2010; Lam *et al.*, 2012a), potato (Högy and Fangmeier, 2009a), barley (Erbs *et al.*, 2010), soybean (Lam *et al.*, 2012b) and vegetables like carrot, radish and turnip (Azam *et al.*, 2013).

Seed germination in response to elevated CO₂ was been reported to decrease in some studies (Ziska and Bunce, 1993; Farnsworth and Bazazz, 1995; Andalo *et al.*, 1996; Quaderi and Reid, 2005), while other reported no change (Huxman *et al.*, 1998; Steinger *et al.*, 2000; Thürig *et al.*, 2003; Thomas *et al.*, 2009; Way *et al.*, 2010) or increase (Wulf and Alexander, 1985; Ziska and Bunce, 1993; Edwards *et al.*, 2001). Increased atmospheric CO₂ concentrations might be expected to increase seed mass because of increased plant assimilate availability (Jablonski *et al.*, 2002), but the effects are highly variable among plant species (Miyagi *et al.*, 2007; Hikosaka *et al.*, 2011). Different studies have reported increase in seed mass (Dijkstra *et al.*, 1999; Steinger *et al.*, 2000; Jablonski *et al.*, 2002; Quaderi and Reid 2005), no change (Edwards *et al.*, 2001; Prasad *et al.*, 2002) or decrease (Huxman *et al.*, 1998; Smith *et al.*, 2000; Wagner *et al.*, 2001) in response to elevated CO₂.

Elevated CO₂ can affect plant fitness, not only through its effects on seed production but also by altering the quality of seeds (morphology, nutrition), therefore affecting germination and seedling performance (growth rate). Elevated CO₂ has been reported to increase the C:N ratio in seeds (Steinger *et al.*, 2000; Jablonski *et al.*, 2002). Hikosaka *et al.* (2011) suggested that seed mass could only increase when N becomes further available at elevated CO₂ concentrations. Carbon content of plant evidently increased with atmospheric CO₂ enrichment and caused subsequent reduction in N concentration in plant tissues (Cotrufo *et al.*, 1998; Gifford *et al.*, 2000; Thuerig *et al.*, 2003). But contradictory findings are also reported for some essential nutrient elements. Zeng *et al.* (2011) reported increase in P and K concentrations in both C₃ (rice) and C₄ (weed *Echinochloa crusgalli*) under elevated CO₂ level (580 µmol mol⁻¹) with increasing uptake of C, N, P, K by rice.

In regions where legumes are a significant component of the human diet, mineral deficiencies (especially Fe and Zn) are quite prevalent (Wang *et al.*, 2003). Idso and Idso (2001) reviewed a number of studies aiming to examine the effects of elevated CO₂ produced food material composition and found variable effects of CO₂ enrichment on the composition of a specific nutrient element. In their OTC study, Manderscheid *et al.* (1995) reported that the changing status of nutrient use efficiency under elevated CO₂ may vary from one crop to other. As for spring wheat (*Triticum aestivum* L.), they documented the increase in P and K with subsequent decrease in Ca and Mg as well as most of the micronutrients (mostly Fe and Zn). However, Grotenhuis and Bugbee (1997) found elevated CO₂ had no effect on either the macro- or micro-nutrient concentration in wheat grains. Contrasting response was reported in spring barley (*Hordeum vulgare* L.) with an overall decrease in nutrient use efficiency under elevated CO₂ although the nutrient harvest index remains unchanged for all nutrients (Ca, K, Mg, N, P, Fe, Mn, and Zn) except S. Decrease in the P, Zn, Fe and Cu concentrations of brown rice but increased Ca concentration with doubling of atmospheric CO₂ was reported by Seneweera and Conroy (1997). In another study, Ziska *et al.* (1997) reported no change in Ca or K concentration in grains regardless of air temperature for Indica rice IR72. Loladze (2002) in his summarized review concluded that CO₂ enrichment across the globe would accelerate the micronutrient (e.g., Cu, Zn and Fe) deficiencies in grains of major

crops, and thus intensify the acute problem of micronutrient malnutrition. No dilution effect on the concentration of micronutrients (e.g., Zn, Mn, Fe, Cu, B, Mo and Se) or macronutrients (e.g., P, K, Mg and S) other than N for field-grown rice grains (Lieffering *et al.*, 2004), no change in concentrations of Zn and Fe and significant decrease in Cu concentration in rice grains (Yang *et al.*, 2007), and no effect on macronutrient (N, P, K, Ca, Mg) composition in potato tuber (Piikki, *et al.*, 2007) were also reported. In contrast, Högy and Fangmeier (2009b) reported a decreasing trend of potato tuber quality with significant reduction of K and Ca. Högy *et al.* (2010b) reported significant decrease in S, B and Cd concentrations in seeds of *Brassica* with decreasing trend for Zn and Mn under elevated CO₂ level. A clear tendency of quality deterioration of rice grains under elevated CO₂ caused serious deterioration of processing suitability, increasing chalkiness, with no change in other macro- and micro-nutrients concentration (Wang *et al.*, 2011). Decreasing concentrations of Ca, Mg, Fe and Co, with increase in B content were reported in spring wheat (*Triticum aestivum*) (Högy *et al.*, 2013). Mishra *et al.* (2012) reported major influence of Zn concentration in roots cells on limited growth stimulation under elevated CO₂ for both low and high B scenarios. With changes in composition of essential nutrient elements, compositions of biological macromolecules in plant system i.e. N-rich protein, C-rich carbohydrates are likely to get modified due to higher concentrations of atmospheric CO₂ (Poorter *et al.*, 1997).

In contrast to the findings by Blumenthal *et al.* (1996) about a possibility of reduction in quality of bread wheat due to lowered protein content under elevated CO₂ levels of (700 µl/L), Raja Reddy *et al.* (2005) reported no deleterious effects on fiber quality and yield of cotton under elevated CO₂ if N supply is optimum. A different response of grain protein concentration to elevated CO₂ was observed for the same crop. In another OTC study on field-grown tropical rice (*Oryza sativa* L.), 200 ppm increment of CO₂ from normal concentration had no effect on grain protein concentration, but with another additional increment of 100 ppm, a reduction in rice grain protein was observed (Ziska *et al.*, 1997). Jablonski *et al.* (2002) found that under elevated CO₂, seed N was significantly reduced for wheat and barley, but not for soybean or rice. Contrary to no effect of CO₂ enrichment on protein content in wheat (Saebo and Mortensen, 1996; Lam *et al.*, 2012a), Oat (Saebo and Mortensen,

1996) and peanut (Burkey *et al.*, 2007), reports are available showing significant decrease in protein content in several field crops, like, barley, rice, wheat, soybean and potato (Lieffering *et al.*, 2004; Högy and Fangmeier, 2008; Wang *et al.*, 2011). Piikki *et al.* (2008) reported increase in the protein yield with significant reduction in grain protein concentration of spring wheat under elevated CO₂. Högy *et al.* (2010b) postulated that CO₂ effect of significant decline in protein concentration in *Brassica napus* cv. *Campino* seeds may be compensated by increased seed yield on total production basis.

Manderscheid *et al.* (1995) reported decrease in the concentration of amino acids in wheat grains while Högy and Fangmeier (2009b) found a significant negative relationship between CO₂ treatment and amino acids with a decrease in protein in potato tubers with increasing CO₂. Decreasing trend in proline content of spring wheat under atmospheric CO₂ enrichment was also reported (Högy *et al.*, 2010a).

Marginal increase in wheat grain starch (Tester *et al.*, 1995), with greater amounts of reducing sugars in carrot, radish and turnip (Azam *et al.*, 2013) and no difference in sucrose concentration in tomato (Islam *et al.*, 1996) were reported. Donnelly *et al.* (2001) found that the starch content of potato tubers and the viscosity of the resulting paste increased under elevated CO₂ levels of 550 and 680 $\mu\text{mol mol}^{-1}$. Picon-Cochard *et al.* (2004) noted an increase in water soluble sugar of bulk forage crops under 600 $\mu\text{mol mol}^{-1}$ of CO₂. Contrasting results are also available, where it is often not greatly altered (Tester *et al.*, 1995; Blumenthal *et al.*, 1996). Increase in starch content was reported in wheat grains (Rogers *et al.*, 1998; Fangmeier *et al.*, 1999; Wu *et al.*, 2004; Högy and Fangmeier, 2008). Sasaki *et al.* (2007) reported that more partitioning of photosynthates produced during both vegetative and grain-filling stages of rice to the grains under atmospheric CO₂ enrichment. Increasing levels of total reducing carbohydrates in potato tuber (Högy and Fangmeier, 2009b) and sugar beet (Manderscheid *et al.*, 2010) was observed under CO₂ level of 550 $\mu\text{l l}^{-1}$. In spring wheat, significant increase in sugars and sugar derivatives under atmospheric CO₂ enrichment was also reported (Högy *et al.*, 2010a).

Literature reviews indicated effect of elevated CO₂ on yield and quality traits have been mostly studied on cereals and tuber crops. On the contrary such reports on legumes are meagre. Legumes play a major role in nutritional balance of food, making it an inseparable component in the food and nutrition security, especially in relation to global climate change mediated by elevated CO₂. Chickpea [*Cicer arietinum* L.] is an important winter-season pulse and the cheapest source of protein for billions of people in developing countries (Joshi *et al.*, 2002). It has a share of 14.6% in global pulse production and grows on 15.3% of the total global pulse area (FAOSTAT, 2010). Chickpea is a good source of protein and carbohydrate and its protein quality is better than many other legumes such as pigeon pea, black gram and green gram (Kaur *et al.*, 2005). The term ‘seed quality’ is used in practice to describe the overall value of a seed lot for its intended purpose (Hampton *et al.*, 2012), and includes the components of species and cultivar purity, seed mass (size), physical purity, germination, vigour, moisture content and seed health. A major challenge ahead for those involved in the seed industry is to provide cultivars that can maximize future crop production in a changing climate for future elevated CO₂ world (Ainsworth *et al.*, 2008; Ceccarelli *et al.*, 2010). There is, therefore, an urgent need to make comprehensive study on the effect of elevated CO₂ on yield quality traits of chickpea. A 2-year open-top-chamber experiment was thus planned to test the hypothesis: a environment of elevated CO₂ may produce seeds with increasing amounts of biological macromolecules (carbohydrates, lipids and protein), for better nourishment during early seedling growth, that may have profound effects on germination and may cause a nutritional imbalance, if taken as food due to compositional changes in stored material in chickpea grains.

8. 2 Materials and Methods

8.2.1 Site description

The open-top chambers (OTC) experiment was conducted on a fairly leveled topography for Pusa- 1105 chickpea genotype, during *rabi* season (mid November-mid April) of 2010-11 and 2011-12, at Indian Agricultural Research Institute, New Delhi, located at 28°35' N latitude, 77°12' E longitude and at an altitude of 228.16 m above mean sea level.

8.2.2 Climate and soil

The site climate was characterized by a typical semi-arid with dry hot summer and mild winters (an average annual rainfall of 710 mm, of which 80 per cent is received during southwest monsoon from July to September). The mean daily maximum and minimum air temperature varies between 10-29 °C and 2-12 °C respectively. The mean daily evaporation (USWB open pan evaporation) was 3.3 mm/day during the crop growth period.

Soil of the study site belongs to *Typic Haplustept* of upper Indo-Gangetic Plain. The surface soil (0-30 cm) of the experimental site was non-calcareous, slightly alkaline in reaction. The site has sandy loam in texture having 68.8 % sand 19.2% silt and 12.2 % clay, with medium to weak angular blocky structure. The average saturated hydraulic conductivity 1.17 cm h⁻¹; pH (1:2.5 soil/water suspension) 7.9; EC 0.64 dS m⁻¹; Soil organic C, 3.7 g kg⁻¹; total N 0.031%; and available P (Olsen) and K (1N NH₄Ac), 5.046 and 453.86 kg ha⁻¹ respectively along with calcium (Ca) content; 59.2 mg kg⁻¹, Magnesium (Mg); 46.1 mg kg⁻¹, S content: 16.9 mg kg⁻¹ and B (hot water) content of 0.56 mg kg⁻¹. Average available cationic micronutrient status of the site soil includes, diethylene triamine pentaacetic acid (DTPA)-extractable Zn, Fe, Cu, and Mn were as follows (mg kg⁻¹); 2.1, 10.25, 0.84, and 11.7, respectively.

8.2.3 Experimental details

The circular structure of OTCs was fixed in the field and fabricated using aluminum frame as described by Saha *et al.* (2011). In two OTCs, elevated levels of 580 µmol mol⁻¹ CO₂ were maintained from 6:00 AM to 6:00 PM from the crop emergence and the other two OTCs were used as chamber controls without any external supply of CO₂. Chickpea (Pusa 1105) was selected for the study. At the end of the crop growth season final harvesting of the crop was performed to assess the grain yield at harvesting maturity, when the total the entire plant dried out with a yellowish brown appearance. Seeds from were cleaned and freed from broken seeds, dust and other foreign materials.

8.2.4 Determination of seed physical characteristics

8.2.4.1 Test weight and density of chickpea seeds

For economic seed yield, pods were sun dried to measure the test weight for each OTC in 4 replicates. Seed volume was determined by transferring 100 seeds from a fixed height (30 cm) into a 100 ml measuring cylinder containing 50 ml of distilled water. The gain in volume was measured in terms of seed volume. Seed density was calculated as seed weight divided by seed volume (Khattak *et al.*, 2006).

8.2.4.2 Hydration capacity, Hydration index and Swelling index

Hydration capacity was recorded as gain in weight after overnight soaking in distilled water. Hydration index was calculated as hydration capacity divided by original seed weight. Cotyledons (100 seeds) were soaked in 100 ml distilled water in a measuring cylinder and covered with aluminium foil. It was left to soak for 24 h at room temperature ($25 \pm 1^\circ\text{C}$), drained, and excess water was removed using a filter paper (Whatman No. 42). Thereafter, the weight of the swollen seeds was measured and the hydration capacity along with the hydration index was calculated. The swelling capacity was determined as gain in volume after overnight soaking, and swelling index was calculated as swelling capacity divided by original seed volume (William *et al.*, 1983; Kaur *et al.*, 2005).

Hydration capacity (per seed basis) = (Weight after soaking - Weight before soaking) / Number of seeds (N=100)(8.1)

Hydration index = (Hydration capacity / Average weight of one seed)(8.2)

Swelling capacity (per seed basis) = (Volume after soaking - Volume before soaking) / Number of seeds (N=100)(8.3)

Swelling index = (Swelling capacity / Average volume of one seed)(8.4)

8.2.4.3 Determination of seed moisture content

Seed moisture was determined in 4 replicates by oven drying seeds at 95°C to constant weight (Walters, 1998). Seed moisture content was expressed as g water g^{-1} dry weight.

Moisture content (%) is calculated as $[(W_1 - W_2) / (W_2)] \times 100$ (8.5)

where, W_1 is initial weight of the seed (g) and W_2 is the final weight of the seed after drying (g).

8.2.4.4 Seed leachate conductivity

25 seeds in 4 replicates were soaked in 25 ml of distilled water at 25°C for 4h along with a control (only distilled water without seeds). The seed leachate was decanted off in clean 50 ml beaker. The electrical conductance of the leachate was measured at room temperature (ISTA, 1985) using digital conductivity meter (Systronics India).

8.2.4.5 Germination percentage

The germination of the seeds was determined by using between paper method (ISTA, 1985). One hundred seeds from each OTC were collected in 4 replications, each of 25 seeds were placed between two layers of moist germination papers. Then the germination papers were rolled carefully ensuring that no excess pressure is placed on the seeds. These were wrapped in a sheet of wax paper to reduce surface evaporation and placed in the germination incubator at 20°C for chickpea in an upright position. After 8 days, the germinated seeds were evaluated for normal, abnormal seedling, fresh un-germinated and dead seeds. Germination percentage was calculated on the basis of normal seedling only.

8.2.4.6 Seedling growth and seedling vigour

Ten normal seedlings from each replicate were taken at random per OTC during both the years of experimentation and the shoot length was measured by linear scale. Seedlings were dried overnight at 70°C temperature and the weight of 10 seedlings together per replicate was measured. Seedling vigour was calculated following Abdul Baki and Anderson (1973) as

Vigour index I = Germination % x Seedling length (Root +Shoot)(8.6)

Vigour index II = Germination % x Seedling dry weight (Root +Shoot)(8.7)

8.2.4.7 Speed of Germination

Twenty seeds in 4 replicates from each OTC were placed on a moistened filter paper in petri dish and kept in an incubator at 20°C. Daily germination count was taken till no more seeds germinated (Vashisth and Nagarajan, 2010). The speed of germination (X) was calculated following Maguire (1962) as:

$$X = \sum(n/t) \quad \text{.....(8.8)}$$

Where n is the number of seeds newly germinating at time t, and t is days from sowing.

8.2.4.8 Enzymes activities assessment related to germination

Enzymes related to the germination process were assessed with germinating chickpea seeds, imbibed in distilled water at 25±1°C for 24 hours.

8.2.4.8.1 Assay of dehydrogenase

Dehydrogenase activity was estimated following the method described by Kittock and Law (1968). The germinating seeds of chickpea were taken in quadruplicate in sample test tubes. Seeds were bisected completely through the mid-section of the distal half; 1 ml of 1% tetrazolium solution was added. The sample tube was incubated in dark at 30°C for 2 hour. The excess solution was drained out and washed thoroughly in distilled water. The seeds were soaked in 9 ml of methyl cellosolve overnight with shoaking till the extraction of red coloured formazan was completed. The extract was decanted and intensity of colour was read as absorption at 480 nm in double beam UV-VIS spectrophotometer. Methyl cellosolve was used to set the absorption at zero.

8.2.4.8.2 Assay of acid and alkaline phosphatase

Acid and alkaline phosphatase activity was measured as given in Sadasivam and Manickam (2008). One gram of germinating seed material was homogenize in 10 ml of ice-cold 50mM citrate buffer (pH 5.3) in a pre-chilled pestle and mortar and filtered through four layers of cheese cloth. The filtrate was centrifuged at 10,000g for 10 min. the supernatant was used as enzyme source. 3 ml of substrate solution (1.49 g EDTA, 0.84 g citric acid and 0.03 g *p*-nitrophenyl phosphate in 100 ml water

and adjusted to pH 5.3) was incubated at 37°C for 5 min. 0.5 ml enzyme extract was added and mixed well. Immediately 0.05 ml was removed and mixed with 9.5 ml of sodium hydroxide 0.085N. This corresponds to zero time assay (blank). The remaining solution (substrate + enzyme) was incubated for 15min at 37°C. Then 0.5 ml sample was drawn and mixed it with 9.5 ml sodium hydroxide solution. The absorbance was measured for both blank and incubated tubes at 405nm in double beam UV-VIS spectrophotometer.

Standard curve preparation

0.2 to 1.0 ml (4 to 20 mM) of the standard (69.75 mg *p*-nitrophenol and dissolved in 5.0 ml distilled water) was taken and diluted to 10.0 ml with NaOH solution. The color was read at 405 nm and the standard curve was drawn. Alkaline phosphatase functions optimally at about pH 10.5. The assay procedure was similar to that for acid phosphatase, except for the substrate solution. The substrate solution was prepared by dissolving 75mg glycine, 10mg magnesium chloride, 165mg *p*-nitrophenyl phosphate in 42 ml of 0.1N sodium hydroxide. The solution was diluted to 100ml, with adjusting the pH at 10.5.

The protein content of the supernatant was estimated by Lowry's method (1951) with bovine serum albumin (BSA) as reference standard. Both of the enzyme activities were expressed as mg protein/g fresh weight. The value of enzyme activity was expressed in terms of mmol *p*-nitrophenol released $\text{min}^{-1} \text{mg protein}^{-1}$.

8.2.4.8.3 Assay of protease

Protease activity was estimated as described by Vashisth and Nagarajan (2010). 1g of germinating seeds were ground in 5 ml 0.2M-phosphate buffer (pH=7.6) by mortar and pestle placed in ice bucket and centrifuged at 12,000 rpm for 30 min at 4°C. Supernatant was decanted in 20 ml centrifuge tube. 0.1 ml of enzyme extract was taken and the volume was made 1 ml by adding phosphate buffer. Sample tube was pre-incubated at 50°C for 10 min. 1 ml of casein (1%) solution was added in all the tubes and incubated at 50°C for 2 hour. The reaction was stopped by adding 3 ml of 5% Trichloroacetic acid solution and centrifuged at 15,000 rpm for 10 min at 4°C.

Supernatant was collected and estimated by Lowry's method with BSA as reference standard. The activity was expressed as mg/ g fresh wt.

8.2.5 Determination of flour characteristics of chickpea

8.2.5.1 Grain sample preparation

Matured pods (per plant basis) were collected in 6 replicates from each OTC, dried at room temperature, grains were separated and soaked in distilled water at room temperature of $25\pm 1^{\circ}\text{C}$ for 10h and manually dehulled (Adebowale and Lawal, 2004). Then the cotyledons were dehydrated overnight in an oven prior to grinding treatment (Boye *et al.*, 2010), to obtain homogenate flours of chickpea seeds by electric seed blender made up of stainless steel and then passing it through a 20-mesh sieve. The flour was stored in a dark chamber at 4°C in airtight glass containers, until use for further functional and biochemical analysis. A part of collected seeds from each replication were dehulled and cotyledons were stored intact separately in polythene bags and used for analysis of different functional properties (minimum cooking time and water uptake ratio).

8.2.5.2 Compositional analysis of biological macromolecules of chickpea flour

Crude protein content was calculated using a factor of 6.25 (Clemente *et al.*, 1999) with the N content determined by micro-Kjeldahl method (Bremner, 1960), while crude lipid was determined by employing the Soxhlet apparatus (AOAC, 1990). Briefly, a piece of paper was folded in such a way to hold the seed meal and wrapped around a second filter paper which is left open at the top like a thimble. A piece of cotton wool was placed at the top to evenly distribute the solvent as it drops on the sample during extraction. The sample packet was placed in the butt tubes of the Soxhlet extraction apparatus and extracted with 2:1 (v/v) chloroform/methanol (~140 drops/min) for 6h without interruption by gentle heating. The extraction flask was allowed to cool and dismantle. The ether was evaporated on a steam or water bath until no odour of ether remains and was cooled at room temperature. The heating process was repeated until constant weight was achieved.

8.2.5.2.1 Total carbohydrate estimation

For the estimation of total carbohydrate by anthrone method (Hedge and Hofreiter, 1962), carbohydrates are first hydrolyzed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxymethyl furfural. This compound forms with anthrone a green coloured product with an absorption maximum at 630nm. 100 mg of the ground sample was weighed into a boiling tube and the material was hydrolyzed by keeping it in boiling water bath for 3 hours with 5 ml of 2.5N HCl and cooled to room temperature. The content was neutralized with solid sodium carbonate until the effervescence ceases and the volume was adjusted to 100 ml and centrifuged. The supernatant was collected and 0.5 and 1ml aliquot was taken out for analysis.

The standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard. '0' served as blank. The volume was adjusted to 1 ml in all the tubes including the sample tubes by adding distilled water, followed by addition of 4 ml of anthrone reagent. The material was then heated for eight minutes in a boiling water bath and was cooled rapidly and the intensity of green to dark green color was read at 630 nm. A standard graph was made by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis. From the graph the amount of carbohydrate present in the sample tube was calculated.

8.2.5.2.2 Reducing sugar estimation:

Reducing sugar was estimated by dinitrosalicylic (DNS) acid method (Miller, 1972). 100 mg of the ground seed sample was weighed and the sugars were extracted with the 5 ml of hot 80% ethanol twice and collecting the supernatant and evaporating it by keeping it on a water bath at 80°C. 10 ml water was added to dissolve the sugars and 0.5 to 3 ml of the extract was taken out by pipette in test tubes with equalizing the volume to 3 ml with water in all the tubes. 3 ml of DNS reagent was added and the content was heated in a boiling water bath for 5 min. When the contents of the tubes were still warm, 1 ml of 40% Rochelle salt solution was added and was cooled followed by reading the intensity of dark red colour at 510 nm. A series of standards were prepared using glucose (0 to 500 mg) and plotted on a graph for calculation.

The amount of reducing sugars present in the sample was calculated using the standard graph.

8.2.5.2.3 Estimation of cellulose

The method described by Updegroff, (1969) was followed for cellulose estimation. In brief, 3 ml acetic/nitric reagent was added to 0.5 g of the ground seed sample in a test tube and mixed in a vortex mixture and the tube was placed in a water bath at 100°C for 30 min. It was then cooled and the contents centrifuged for 15-20 min. The supernatant was discarded and the residue was washed with distilled water followed by addition of 10 ml of 67% sulphuric acid and was allowed to stand for 1 h. 1 ml of the above solution was diluted to 100 ml. To 1 ml of this diluted solution, 10 ml of anthrone reagent was added and mixed well. The tubes were heated in boiling water bath for 10 min. and cooled. The colour absorbance was measured at 630 nm. A blank was set with anthrone reagent and distilled water. 100 mg cellulose was taken in a test tube and got progressed. Instead of just taking 1 ml of the diluted solution, a series of volumes (say 0.4 to 2 ml corresponding to 40-200 µg of cellulose) was taken to develop the colour to be read at 630 nm.

8.2.5.2.4 Estimation of Starch by anthrone reagent

The method described by Hedge and Hofreiter (1962) was followed for determination of starch content in chickpea seeds. In brief, 0.1 g of the ground seed sample from each treatment was homogenized in hot 80% ethanol to remove sugars. Centrifuged and retained the residue. Washed the residue repeatedly with hot 80% ethanol till the washing did not give colour with anthrone reagent. The residue was dried over a water bath. To the residue 5.0 ml of water and 6.5 ml of 52% perchloric acid was added. After extracting at 0°C for 20 min, it was centrifuged and the supernatant was saved.

The extraction was repeated using fresh perchloric acid, centrifuged and pooled the supernatant and volume made up to 100 ml. 0.1 ml was pipetted out of the supernatant and made up the volume to 1 ml with water. The standards were prepared by taking 0.2, 0.4, 0.6, 0.8 and 1 ml in each tube with water. 4 ml of anthrone reagent was added to each tube. After eight minutes of heating in a boiling

water bath, it was cooled rapidly and the intensity of green to dark green colour was read at 630 nm. The glucose content in the sample was evaluated using the standard graph and multiplying the value by a factor 0.9 to arrive at the starch content (Thayumanavan and Sadasivam, 1984).

8.2.5.2.5 Determination of crude fibre

Crude fibre was determined gravimetrically (Maynard, 1970). Exactly 2g of ground material was treated with ether or petroleum ether to remove fat (Initial boiling temperature 35 -38°C and final temperature 52°C). After extraction with ether, 2g of dried material was boiled with 200 ml of sulphuric acid for 30min with bumping chips and filtered through muslin and washed with boiling water until washing were no longer acidic. It was then again boiled with 200 ml of sodium hydroxide solution for 30min. The content was filtered through muslin cloth again and washed with 25 ml of boiling 1.25% H₂SO₄, three 50 ml portions of water and 25 ml alcohol. The residue was removed and transferred to ashing dish (pre-weighed dish W₁) and dried for 2h at 130 ±2°C. The dish was then cooled in a desiccators and weighed (W₂). The content was ignited for 30min at 600 ±15°C, cooled in a desiccators and reweighed (W₃).

$$\% \text{ crude fibre} = \frac{\text{Mass loss on ignition } (W_2 - W_1) - (W_3 - W_1) \times 100}{\text{Weight of the sample}} \quad \dots (8.9)$$

8.2.5.2.6 Proline estimation

Proline content was determined by the method given by Bates *et al.* (1973). 0.5 g of seed material was extracted by homogenizing in 10 ml of 3% aqueous sulphosalicylic acid and filtered the homogenate through Whatman No. 2 filter paper. During selective extraction with aqueous sulphosalicylic acid, proteins are precipitated as a complex. 2 ml of filtrate was taken in a test tube and added 2 ml of glacial acetic acid and 2 ml acid ninhydrin in acidic conditions (pH 1.0) followed by heating it in the boiling water bath for 1h. The reaction was terminated by placing the tube in ice bath. 4 ml toluene was added to the reaction mixture and stir well for 20-

30sec. and the toluene layer was separated and warmed to room temperature. The red color intensity was measured at 520 nm. A series of standard with pure proline was tested in a similar way to prepare a standard curve and the amount of proline in the test sample was calculated from the standard curve.

8.2.5.2.7 Estimation of total free amino acids

Extraction of Amino Acids

500mg of plant sample was weighed and grinded in a pestle and mortar with a small quantity of acid-washed sand. To this homogenate, added 5 to 10 ml of 80% ethanol. Then filtered or centrifuged. The filtrate or supernatant was saved and the extraction was repeated twice with the residue and all the supernatants were pooled into a single one. The volume was reduced by evaporation and the extract was used for the quantitative estimation of total free amino acids. If the tissue is tough, boiling in 80% ethanol for extraction could be used.

Estimation

To 0.1 ml of extract, 1 ml of ninhydrin solution was added and the volume was made up to 2 ml with distilled water. The tube was heated in boiling water bath for 20 min and 5 ml of the diluents was added and mixed the contents thoroughly. After 15 min the intensity of the purple color was read against a reagent blank in a colorimeter at 570 nm. The colour is stable for 1h. The reagent blank was prepared as above by taking 0.1 ml of 78% ethanol instead of the extract.

Standard Preparation

50 mg leucine was dissolved in 50 ml of distilled water in a volumetric flask. 10 ml of this stock standard was taken out and diluted to 100 ml in another flask for working standard solution. A series of volume from 0.1 to 1ml of this standard solution gives a concentration range 10µg to 100 µg. Proceeded as that of the sample and read the colour. A standard curve was drawn using absorbance versus concentration. Find out the concentration of the total free amino acids in the sample was determined and expressed as percentage equivalent of leucine (Misra *et al.*, 1975).

8.2.5.2.8 Determination of total phenols

Exactly 1.0g of the seed sample was weighed and grinded with a pestle and mortar in 10-time volume of 80% ethanol. The homogenate was then centrifuged at 10,000rpm for 20 min at room temperature and the supernatant was collected. Re-extraction was performed for the residue with five times the volume of 80% ethanol, centrifuged and pooled the supernatants (Malick and Singh, 1980). The supernatant was evaporated to dryness and the residue was dissolved in a known volume of distilled water (5 ml). Different aliquots (1 ml) were pipetted out into test tubes followed by volume made up in each tube up to 3 ml with water with subsequent addition of 0.5 ml of Folin-Ciocalteu reagent which consists of 2.5% sodium molybdate and 19% sodium tungstate (Senter *et al.*, 1989). After 3 min, 2 ml of 20% Na_2CO_3 solution was added to each tube and mixed thoroughly. The tubes were placed in boiling water for exactly one min, cooled and measured the absorbance at 650 nm against a reagent blank. The standard curve was prepared using different concentrations of catechol

8.2.5.2.9 Mineral composition analysis of chickpea flour

Homogenate dried flours of chickpea seeds were oven dried at 80°C for 24 hours. Dry ashing was conducted by taking 1 gm of oven dried ground seed material placed in quartz crucible and ignited at 450°C (maximum temperature of 500°C) for 8 h in a muffle furnace in 10 replicates (Miller, 1998). The resulted ash was dissolved with 10.0 ml 1N HCl solution, facilitated by gentle heating on a hot plate (Munter and Grande, 1981). The contents of the quartz crucible were transferred quantitatively into a 50 ml volumetric flask, diluted to 50 ml volume with deionized water, capped, and inverted three times and elemental analysis of plant digests was made using AAS for Ca, Mg, Cu, Zn, Mn, and Fe (Watson and Isaac, 1990). Phosphorus was determined by the phosphovanado-molybdate (yellow) method (AOAC, 1990). B content was determined by azomethane-H procedure (Bingham, 1982). Total S was obtained by acid digest and was prepared by oxidizing each sub-sample with a nitric and perchloric acid (2:1) mixture (Zia-Ul-Haq *et al.*, 2007). Aliquots were used to estimate K by flame photometer (flame photometer model-EEL) and S for neplalometric measurement. All the samples were quantified against standard

solutions of known concentration that were analysed concurrently and samples having analyte concentrations exceeding the highest value of standard were diluted for reanalysis.

8.2.5.2.10 Functional properties of chickpea seeds

The functional properties have been defined as “those physical and chemical properties that influence the behaviour of proteins in food systems during processing, storage, cooking and consumption” (Kinsella and Melachouris, 1976). Functional properties could be classified according to the mechanism of action on three main groups: (i) properties related with hydration (absorption of water/oil, solubility) (ii) properties related with the protein surface (emulsifying and foaming activities) (iii) properties related with the protein structure and rheological characteristics (gelification) (Moure *et al.*, 2006).

8.2.5.2.11 Bulk density of chickpea flour

The flour samples were gently filled into 10 ml graduated cylinders, previously tared. The bottom of each cylinder was gently tapped on a laboratory bench several times until there was no further diminution of the sample level after filling to the 10 ml mark. Bulk density was calculated as weight of sample per unit volume of sample (g/ml). Measurements were made in triplicate for the sample collected for both of the years.

8.2.5.2.12 pH determination

The pH of flour samples was measured using a pH meter. Each flour sample (10 g) was suspended in 100 ml of boiling distilled water. After cooling, the slurry was shaken (1500 rpm, 25°C, 20 min) using an orbital shaker (Angulo-Bejarano *et al.*, 2008) and its pH was determined.

8.2.5.2.13 Minimum cooking time

Minimum cooking time was determined according to the method outlined by Yadahally *et al.* (2009). In brief, about 200 ml of deionized water in 250 ml beaker was boiled in a standard laboratory hot-plate. Approximately 50 g of chickpea seeds (cotyledons) were boiled with the water being added at regular intervals to maintain

the water level constant. Cooking was complete when colour of 80% to 100% of seed (cotyledon) changed completely from whitish yellow to yellow due to gelatinization (Badshah *et al.*, 2003, 2006). The cotyledons were tested after 40 min boiling by removing them from the beaker at every 3 min interval by using a sterile spatula and tested for softness by pressing between finger and thumb. The cooking time was determined based on the time taken. The minimum cooking time was determined on removing a few cotyledons at different time intervals for cooking of raw cotyledon to cook up to the required softness checked by pressing between two glass slides until no uncooked core was left (Seena and Sridhar, 2005).

8.2.5.2.14 Water uptake ratio

Approximately 50 g of seed cotyledons (edible portion) were cooked for minimum cooking time in 250 ml of distilled water. The cooked cotyledons were then removed, drained and subsequently water on the surface was wiped using filter papers. The samples were weighed and the water uptake ratio was calculated as the ratio of weight gained after cooking to weight before cooking (Seena and Sridhar, 2005).

8.2.5.2.15 Water and oil absorption capacity

Water absorption of flour was measured by the centrifugation method of Sosulski (1962). The flour sample (3.0 g) was dispersed in 25 ml of distilled water and placed in pre-weighed centrifuge tubes. The dispersions were stirred occasionally, held for 30 min, followed by centrifugation for 25 min at 3000g. The supernatant was decanted, excess moisture was removed by draining for 25 min at 50 °C, and the sample was reweighed. For the determination of fat absorption, the method of Lin *et al.* (1974) was used. Sample (0.5 g) was mixed with 6 ml of corn oil in pre-weighed centrifuge tubes. The contents were stirred for 1 min with a thin brass wire to disperse the sample in the oil. After a holding period of 30 min, the tubes were centrifuged for 25 min at 3000g. The separated oil was then removed with a pipette and the tubes were inverted for 25 min to drain the oil prior to reweighing. Triplicate determinations were carried out and the water and oil absorption capacities were expressed as grammes of water or oil bound per grammes of the flour sample on oven dry basis.

8.2.5.2.16 Foaming properties

The foaming properties were determined in three replicates for each OTC by employing the method of Seena and Sridhar (2005). Approximately, 250 mg of cotyledon flour was mixed with 250 ml of distilled water and the volume (volume before whipping) was measured by using measuring cylinder. Further, the solution was whipped by using a homogenizer, at high setting for 2–3 min. The blend was immediately transferred into a graduated cylinder and the homogenizer cup was rinsed with 10 ml of distilled water. The volume (volume after whipping) was measured again within 20 Sec. The samples were then allowed to stand for 30 min at room temperature ($25 \pm 1^\circ\text{C}$) and the volume (volume after standing) was measured. The foam capacity (FC) and foam stability (FS) were calculated as follows:

$$\text{FC\%} = \{(\text{volume after whipping} - \text{volume before whipping}) \times 100\} / \text{volume before whipping} \quad \text{.....(8.10)}$$

$$\text{FS\%} = \{(\text{volume after standing} - \text{volume before whipping}) \times 100\} / \text{volume before whipping} \quad \text{.....(8.11)}$$

8.2.5.2.17 Least gelation concentration

Gelling capacity is very useful in food systems such as puddings and sauces that require thickening and gelling (Ma *et al.*, 2011). The least gelation concentration (LGC) of chickpea flour was determined by the method of Sathe *et al.* (1982). Test tubes containing suspensions of 2%, 4%, 6%, 8%, 10%, 12%, 14%, 16%, 18% and 20% (w/v) of material in 5 ml distilled water were heated for 1h in boiling water, followed by rapid cooling under cold running water in two replications. The tubes were further cooling at 4°C for 2h. LGC is defined as the minimal protein concentration required for inverting a tube without producing sliding of the gel in the walls i.e. the concentration above which the sample did not fall down or slip when the test tube was inverted.

8.2.6 Statistical analysis

The experiment was conducted in a completely randomized block design (CRD) with four replications for each ambient and elevated CO_2 treatment. Statistical analysis of

the data was done for two-tailed hypothesis using SPSS statistical software Version 16.0 (SPSS Inc., Chicago).

8.3 Results

Physical attributes of chickpea seeds showed partial influence of elevated CO₂ (Table 8.1a). Although there was no treatment effect in case of seed test weight, seed volume, seed density and moisture content under elevated CO₂, but average hydration capacity was 15.6% higher along with 18.1% increase in hydration index, with concomitant increase of 27.2% for swelling capacity and 28.1% for swelling index under elevated CO₂ in both the year of experimentation ($p < 0.05$).

Seed germination (Table 8.2) was highly affected by elevated CO₂. Significant reduction in germination percentage (22-28%) and speed of germination 26-32% was observed under elevated CO₂ ($p < 0.01$). Although there was no significant difference in root and shoot length, length of the seedlings and dry weight of germinated seedlings between elevated and ambient CO₂, but significant reduction in germination ultimately resulted in decrease in vigour index I and II by 1.3 and 1.4 times ($p < 0.01$), respectively, under elevated CO₂. Seed leachate conductivity showed a significant increase of 10.2% ($p < 0.05$) and 17.1% ($p < 0.01$) for elevated CO₂-exposed seeds during 2010-11 and 2011-12, respectively. The acid and alkaline phosphatase activities showed no significant difference. Viability of chickpea seeds (Fig. 8.1) was evaluated by dehydrogenase activity test, which also showed a sharp decline of 51% during 2010-11 and 35.4% during 2011-12 under elevated CO₂ during both the year of experimentation. The protease activity also reduced by 37.7% and 46.3% ($p < 0.05$) during both the years.

Proximate analysis showed significant changes in macromolecular composition in chickpea flour under elevated CO₂ enrichment (Fig. 8.2a). Significant decrease in oil content by 30% with a concomitant reduction of crude protein content by 8-10% ($p < 0.01$) was observed. A significant increase in ash content by 17.6% in 1st year and 21.9% in 2nd year was recorded, along with significant increase in total carbohydrate content by 13.6% and 11.5% in respective years. Increase in reducing sugar content by 26-28% and starch content by 17-23% were also recorded ($p < 0.01$). Cellulose content in grains decreased by 9.5% during 2010-11 and 10.8% during

2011-12 (both non-significant), while significant reduction of 34% in crude fibre content was noted under elevated CO₂ exposure. Reduction in total phenol content was recorded for atmospheric CO₂ enrichment during both of the years of experimentation (Fig. 8.2b). During 2010-11, the reduction was 16.8%, but reduced by 35% during 2011-12. Proline and total free amino acid contents in chickpea also reduced significantly by 26% and 15%, and 17% and 35% during the two years of experimentation.

Elemental analysis showed increase in grain carbon by 14.4 and 19.1%, but reduction in total N content by 10.2% and 8.4 % during 1st and 2nd years, respectively ($p < 0.01$) (Table 8.3a). Grain C:N ratio showed an average increase of 27.3% and 30.1% in the respective years. Grain P content showed no significant effect of atmospheric CO₂ enrichment. But, grain K was found to be diluted by 33% during 2010-11 and 30.5% during 2011-12 under elevated CO₂, along with a reduction in grain sulphur content by 29.3% and 31.8% ($p < 0.01$) respectively for both the consecutive years. The Ca content in chickpea grains was reduced by 26% and 28% during the experimental years, with an overall reduction in Ca: P ratio by 28.1% under CO₂ enrichment. Reduction in Mg content was non-significant, and so were other micronutrient contents except Zn (Table 8.3b). Among all the micronutrients, Zn was found to be particularly sensitive, which reduced by 39% ($p < 0.01$) under atmospheric CO₂ enrichment.

Physical and functional properties of chickpea flour showed no significant difference in bulk density and pH of the flour suspension, along with cooking time, water uptake ratio for elevated CO₂ treatment as compared to ambient (Table 8.4). A non-significant but reducing trend was found for foaming capacity by 4.7% and foam stability by 7.9% (mean of two years). Significant reduction in oil absorption capacity by 24.5% along with 52.3% increase in water absorption capacity was recorded in elevated CO₂ treatment during first year of experimentation. Similar trend was observed during 2nd year, in which oil absorption capacity decreased by 30.1% with 35.1% increase in water absorption capacity. Least gelation concentration showed a remarkable reduction, from 14% concentration under ambient CO₂ treatment to 10% concentration under elevated CO₂, during both of the year of experimentation (Table 8.5).

8.4 Discussion

A large and variable response in seed mass in response to elevated CO₂ has been reported for different crop species. No significant difference in seed test weight, moisture content, volume and density was observed in this study which is in agreement with Edwards *et al.* (2001) and Prasad *et al.* (2002). Our experimental data on seed physical parameters (Table 8.1), were in agreement with values reported by others, varying between 21.9- 35.69 g for seed test weight, 17 - 27 ml for 100 seed volume, 1.29 – 2 g ml⁻¹ for seed density (Kaur *et al.*, 2005; Khattak *et al.*, 2006; Malik *et al.*, 2011) but slightly lower for seed moisture 7.3- 7.89% (Iqbal *et al.*, 2006; Costa *et al.*, 2006). Theoretical values reported in available literature for *kabuli* chickpea seeds were relatively higher than *desi* chickpea seeds (Kaur *et al.*, 2005) ranging between 0.20-0.37 for hydration capacity; 0.91-1.35 for hydration index; 0.23 – 0.93 for swelling capacity and 1.36 - 3.03 for swelling index (Williams *et al.*, 1983; Malik *et al.*, 2011). Zia-Ul-Haq *et al.* (2007) and Bibi *et al.* (2007) reported similar values for hydration capacity, hydration index, swelling capacity and swelling index in different *desi* chickpea seeds. In our study, significant increase ($p < 0.05$) in hydration capacity, hydration index, swelling capacity and swelling index by 15.6%, 18.1%, 27.2% and 28.1% respectively, for the seeds grown under elevated CO₂. Strong positive correlations of swelling index with swelling capacity ($r = 0.575$, $p < 0.01$) and hydration index ($r = 0.819$, $p < 0.01$) was also noted (Table 8.1b).

Seed germination, the most important criteria for seed industry declined significantly under CO₂ enrichment (Table 8.2). Germination percentage reduced by 65-69% for elevated CO₂ treatment compared to 87-90% under ambient condition. Results are in conformity with those reported by Andalo *et al.* (1996) in *Arabidopsis* and Quaderi and Reid (2005) in canola, but Steinger *et al.* (2000) found no change for *Bromus erectus*. Water absorbing capacity of seeds depends on cell wall structure, composition of seed and compactness of the cells in the seed (Muller, 1967). Sefa-Dedah and Stanley (1979) reported that seed size and seed coat thickness play an important role in the water absorption characteristics of legume seeds. The increase in water absorption may be attributed to increased seed coat permeability and soft seed cotyledons for the seeds grown under atmospheric CO₂ enrichment. Moreover, increased seed leachate conductivity was also the indicator of reduced

seed coat membrane integrity of the seeds (Vashisth and Nagarajan, 2010) as increased plasma membrane permeability results in higher solute leakage and was reported to be a coinciding factor for loss in seed germination (Golovina and Tikhonov, 1994). Furthermore, the speed of germination (Table 8.2) also reduced significantly along with a sharp reduction in associated enzyme activity with have major influence on seed germination process (Fig. 8.1). In addition to decreased seed dehydrogenase activity, a significant decline of protease activity is a strong indicator of reduced proteolytic activity and protein degradation during the germination process (Basha and Beevers, 1975). All of these reiterate reduced seed germination under elevated CO₂. The activities of phosphate solubilizing enzymes like acid and alkaline phosphatase did not differ significantly between elevated and ambient CO₂ treatments, although the numerical value was in agreement for acid phosphatase (Bhakuni *et al.*, 2009) but with a relatively lower value for alkaline phosphatase activity in chickpea seeds (Salgado *et al.*, 2001). In totality, this indicates no change in the activity of enzymes involved in phosphate metabolism (Fincher, 1989). This confirmed that the reduced seed viability is a major factor for reduced seed germination and development process for the seed sourced under elevated CO₂ exposure (Hampton *et al.*, 2012).

Huxman *et al.* (1998) reported that the seedlings grown under elevated CO₂ had a reduced relative growth rate and achieved smaller final mass over the same growth period due to smaller seed reserves than ambient seeds of *Bromus rubens*. In our experiment, although seedling performance (root length, shoot length, length of the seedlings and the dry weight of the newly emerged seedlings) was not found to be significantly influenced by elevated CO₂ treatment, considerable decrease in vigour index I and II could be attributed to reduced germination percentage. No effect for elevated CO₂ was reported by Edwards *et al.* (2001), but greater root length with no effect on tissue biomass for the seedlings grown from elevated CO₂ seed) was reported by Hussain *et al.* (2001).

A sharp variation in biological macromolecular composition in chickpea seeds was observed under elevated atmospheric CO₂ (Fig. 8. 2a). Values reported in the literature for *kabuli* chickpea fall within the ranges of 2.7-3.72% for ash content, 16.6– 28.2% for crude protein content (Ereifej *et al.*, 2001; Singh *et al.*, 2004; Alajaji

and El-Adawy, 2006; Boye *et al.*, 2010), 5.88 – 7.0% for oil (Ereifej *et al.*, 2001; Singh *et al.*, 2004; Alajaji and El-Adawy, 2006) and 59.9 – 70.8% total carbohydrate (Hedley, 2001; Singh *et al.*, 2004; Jukanti *et al.*, 2012), along with 29.8 -32.8% starch content (Ereifej *et al.*, 2001), 1.1–14.2% total soluble sugars (Hedley, 2001; Maheri-Sis *et al.*, 2008; Sreerama *et al.*, 2012; Jukanti *et al.*, 2012), 3.3 -6.4% for crude fibre (Sotelo and Adsule, 1996; Ereifej *et al.*, 2001; Alajaji and El-Adawy, 2006) and 1.1–13.7% cellulose (Salgado *et al.*, 2001; Hedley, 2001). In further conformity to these, sharp increase in grain carbohydrate (i.e. reducing sugar, starch, and total carbohydrate) and reduction in grain protein content are attributed to elevated CO₂-induced increased transportation of photosynthetic assimilates towards the grain fraction (Wheeler *et al.*, 1996) causing reduced demand for nitrogen (Fangmeier *et al.*, 1997). This has a direct negative impact on grain protein accumulation (Högy and Fangmeier, 2008; Taub *et al.*, 2008; Pleijel and Uddling, 2012; Fernando *et al.*, 2012a). Reduction in total free amino acid content (Fig. 8. 2b) in chickpea grains is also supported by previous findings (Högy *et al.*, 2009c). Our experimental values for total phenol content (2.02-2.47%) in grains were slightly higher than reported values (Salgado *et al.*, 2001; Nithiyanantham *et al.*, 2012). Reduced level of phenolic compounds accumulation in the chickpea seeds, grown under elevated CO₂, indicated partial improvement in seed quality, due to less accumulation of lignin and reactive free radicals with sufficient B supply (Bhakuni *et al.*, 2009). Crude fibre largely comprises of lignin and cellulose constituting 97% of totality plus mineral matter. Crude fibre represents 60-80% of total cellulose and 4-6% of total lignin (Sadasivam and Manickam, 2008). Grain cellulose content showed a non-significant but consistent reduction; along with significant reduction in crude fibre content under elevated CO₂ treatment (Azam *et al.*, 2013). Thus, there may be a possibility of reduced lignin content that had a greater chance to occur in chickpea grains under elevated CO₂. The grain proline content values agreed with those reported by Wood and Grusak (2007). Accumulation of proline occurs in cell in response to water deficit to protect protein structures as cell dehydrater and also it acts as an organic N source. Dehydration tolerance is related to the degree of osmotic adjustment (Hsiao *et al.*, 1984). In agreement with Högy *et al.* (2010a), a significant decrease in seed proline content was recorded, which implies lower water stress under elevated CO₂ treatment.

Allen and Boote (2000) revealed that legumes may use increased C gain under elevated CO₂ for increased N fixation, and can therefore increase seed mass without decreasing seed N (Hampton *et al.*, 2012). In contrast to this, a sharp increase in grain C:N ratio (Table 8.3a) was recorded under elevated CO₂ in this study. This is attributed to an average 16.7% ($p < 0.01$) increase in grain carbon along with 9.3% ($p < 0.01$) decrease in grain N (Steinger *et al.*, 2000) during both the years. Miao (1995) found that seed size acted synergistically with atmospheric CO₂, acting as a very strong C sink to enhance seedling growth. Increased C sink in seed material is likely to enhance photosynthetic production with further improvement in the energy reserves of seed. The increment in total carbohydrate, starch and other reducing sugar content in seeds from plants grown under elevated CO₂ supports the findings. But reduction in N content in chickpea grains due to elevated CO₂ (dilution effect) was not compensated by increased atmospheric N₂ fixation (data not presented) by leguminous chickpea plants under elevated CO₂ environment. Thus, elevated CO₂ could enrich the grain C in chickpea seeds, by making it more carbonaceous, with low grain N as well as total protein content.

Our result of no change in grain phosphorus concentrations under elevated CO₂ is supported by Fangmeier *et al.* (1997) and Högy *et al.* (2009c), although decrease in P has also been reported (Fernando *et al.*, 2012b). Our range of P concentration in chickpea grains coincided with the range as suggested by Ereifej *et al.* (2001) and Jukanti *et al.* (2012). No change in phosphatase and alkaline phosphatase activity in grains from elevated CO₂ also supported our results. Concentration of mineral in seeds varied in response to both genetic and environmental factors (Wang *et al.*, 2003). K concentration (25.6-31.1 mg g⁻¹) in the chickpea grain was slightly higher than those reported earlier (Ibáñez *et al.*, 1998; Ereifej *et al.*, 2001; Alajaji and El-Adawy, 2006; Adak *et al.*, 2007; Jukanti *et al.*, 2012). Our experimental data on nutrient contents in chickpea seeds (Table 8.3a and b) revealed close similarity with the values reported in literature, viz. 0.97-3.4 mg g⁻¹ for Ca and 0.61-2.4 mg g⁻¹ for Mg (Ibáñez *et al.*, 1998; Ereifej *et al.*, 2001; Adak *et al.*, 2007; Jukanti *et al.*, 2012). Regarding cationic micronutrients (Table 8.3b), it ranged between 5.32-26.6 µg g⁻¹ for Cu, 18.03-44.0 µg g⁻¹ for Zn, 25.57 -126 µg g⁻¹ Fe and 14.83-60.3 µg g⁻¹ for Mn respectively (Ibáñez *et al.*, 1998; Ereifej *et al.*, 2001; Alajaji and El-Adawy, 2006;

Adak *et al.*, 2007; Jukanti *et al.*, 2012). For B, it was within the sufficiency range of 8.6-21.4 $\mu\text{g g}^{-1}$ as reported by Bhakuni *et al.* (2009) along with S content in chickpea ranging between 1.6 - 2.2 mg g^{-1} (Pettersson *et al.*, 1997). In support of the findings of reduced S, Ca, Mg, Fe, Zn, Cu, and Mn concentration (Fernando *et al.*, 2012b; Högy *et al.*, 2013) in wheat grain, Högy *et al.* (2010b) reported reduction in seed quality in oilseed rape due to reduction in Zn and S content under elevated CO_2 . We also found a sharp reduction ($p < 0.01$) in K, Ca, S, Zn concentration by 31.7, 27.1, 30.6 and 38.5% respectively. Although no significant changes were detected for Mg, Cu, Fe, Mn and B content in chickpea seeds, reduction in N, K, Ca, S and Zn concentration along with increased C accumulation suggested possibility of nutritional imbalance in chickpea seeds. Therefore, advantage of increased C content due to increased carbohydrate accumulation at elevated CO_2 was offset by the reduced N concentration and crude protein content, and also other micronutrient contents (Steinger *et al.*, 2000). Increase in C:N ratio (Huxman *et al.*, 1998; Thürig *et al.*, 2003) can lead to a reduction in the ability of the seed to supply the amino acids required for the *de novo* protein synthesis necessary for embryo growth in the germinating seed (Hampton *et al.*, 2012). This could not be ameliorated by the elevated CO_2 induced carbohydrate enrichment in chickpea seeds, and ultimately reduced seed viability. This also confirms the negative effect of atmospheric CO_2 enrichment on seed germination process and seed quality (Andalo *et al.*, 1996).

On the nutritional security front, CO_2 enrichment can have far-reaching implications. While grain protein content along with free amino acid content was reduced due to elevated CO_2 making chickpea grain more carbonaceous, modification in our daily prescribed balanced diet may be recommended. Being an important factor for balanced nutrition, the values of Ca: P ratio obtained from our experiment were in agreement with other published literatures for chickpea (Hadjipanayiotou and Economides, 2001; Ereifej, *et al.*, 2001). In comparison to cereal grains, legumes contain higher Ca than P and their ratio is usually more than 1.0 (Iqbal *et al.*, 2006). Although the average P content remained unaffected, elevated CO_2 -induced dilution of Ca concentration by 28.1% in chickpea grains resulted in a net reduction of Ca: P ratio under elevated CO_2 exposed chickpea grains. Thus, it has a potential to aggravate nutritional imbalances in future

(projected) elevated CO₂ world. Mineral supplementation in diet may be an effective alternative approach to correct this situation (Zia-Ul-Haq *et al.*, 2007).

A moderate effect of atmospheric CO₂ enrichment was recorded on chickpea flour quality (Table 8.4). Flour bulk density (0.41- 0.571 g ml⁻¹) and pH (5.9-6.3) values agreed with others (Kaur and Singh, 2005; Angulo-Bejarano *et al.*, 2008). No significant effect of atmospheric CO₂ enrichment on flour physical and cooking properties were observed. The varietal influence in cooking quality was reported to exist even in the same legume (Narasimha and Desikachar, 1978). The cooking properties of chickpea seeds are very important as it is mostly consumed after boiling/cooking (Badshah *et al.*, 2003). In the present study, the minimum cooking time required was about 94-102 minutes. No significant difference between cooking time and water uptake ratio of seeds was observed between elevated and ambient CO₂ and can be explained by unchanged flour bulk density under elevated CO₂ (Badshah *et al.*, 2003, 2006). But some of other functional properties, like water and oil absorption characteristics, gelation, were significantly influenced by elevated atmospheric CO₂.

Water absorption characteristics represent the ability of a product to associate with water under moisture limited conditions. Values of water absorption capacity were ranging between 1.13-1.23 g/g, slightly less than reported values of 1.33 g/g for chickpea (Kaur and Singh, 2005). Significant increase in water absorption capacity may be attributed to more hydrophilic constituents, such as polysaccharides (Hodge and Osman, 1976), to be present in the chickpea grains grown under elevated CO₂. Starch, being only storage polysaccharides and the principal carbohydrate constituent of chickpea (Wood and Grusak, 2007) increased in chickpea seeds under elevated CO₂. This might explain increased water absorption characteristics, along with possible increase in polar side chains in flour protein molecules obtained from chickpea grain that bind to water (Sridaran *et al.*, 2012).

The oil absorption capacity is an important functional property that is negatively correlated with water absorption capacity. It relies mainly on its capacity to physically entrap oil by a complex capillary attraction process (Sreerama *et al.*, 2012), as it is acting as a flavour retainer that helps to improve the mouth feel

(Kinsella and Melachouris, 1976; Khattab *et al.*, 2009), improving palatability and extension of shelf-life (Adebowale, and Lawal, 2004). Higher oil absorption capacity value of chickpea flour has been reported earlier (Kaur and Singh, 2005; Boye *et al.*, 2010). In this study, fat absorption capacity varied between 1.23 to 1.35 g/g for Pusa-1105 genotype of chickpea, which was slightly lower than the reported values of 1.76 g/g (Ma *et al.*, 2011), but much higher than the value of 1.09 g/g reported by Sreerama *et al.* (2012). In addition to the findings of a significant reduction in crude protein content by 9.3% ($p < 0.01$), our result of a significant reduction in oil absorption capacity indicates reduction in nonpolar side chains in flour protein molecules, resulting reduced hydrophobic properties of proteins and the inferior fat-binding performance of non-polar amino acid side chains (Kinsella and Melachouris, 1976) under elevated CO₂ exposure.

Foaming properties (foaming capacity and stability) were not influenced by atmospheric CO₂ enrichment. Carbohydrate addition (Sathe *et al.*, 1982; Wang and Kinsella, 1976) was found to be responsible for increased foaming capacity and stability (Adebowale, and Lawal, 2003), coupled with reduction in different protein concentration (Adebowale and Lawal, 2004) of different legume protein concentrate. Our findings of non-significant reduction in foaming capacity and foam stability can be explained by significant increase in starch and reducing sugar content in chickpea grains along with significant reduction in grain protein content under elevated CO₂.

Gel can be defined as an intermediate interactive state between liquid water and solid molecular net, formed by proteins, polysaccharides or by a mixture of both. Gelation may be described as a process in which denatured molecules cross-link to form aggregates stabilized by a variety of bonds including, electrostatic interactions, hydrogen, hydrophobic and/or disulfide bonds (Ma *et al.*, 2011). Partial denaturation due to unfolding of the tertiary protein structure enables it to form gel more efficiently than carbohydrate (Moure *et al.*, 2006). Sathe *et al.* (1982) attributed variations in the gelling capacity of various pulse flours to different relative ratios of constituents such as proteins, carbohydrates, and lipids as well as the interactions between all of the constituents making up the pulses. Least gelation concentration (LGC) is a measure of the ability of proteins to form gels. A lower value of LGC is an indicator of more gelling capacity (Sridaran *et al.*, 2012). We found a significant

reduction in LGC value from 14% for ambient CO₂ exposed seeds to 10% for elevated CO₂ exposed seeds, with a sharp reduction of protein solubility and crude protein content by 9.3% ($p < 0.01$). Kaur and Singh (2005) reported least gelation concentration (LGC) for various chickpea flours ranged from 10% to 14%. Flour from *kabuli* chickpea cultivar was found to form a relatively firm gel, at a significantly lower concentration (10%) relative to *desi* chickpea flours. Our result of forming firm gel of chickpea flours stood around a concentration of 15% (w/v) that was similar to that reported by Ma *et al.* (2011). Least gelation capacity reduced with the addition of maltose, lactose, sucrose and starch (Bryant and Mc Clements, 2000; Adebawale and Lawal, 2004; Sridaran *et al.*, 2012). Turgeon and Beaulieu (2001) also reported the improvement in protein gel texture using carbohydrates. Being a major source of protein, chickpea was reported to contain more carbohydrates than other legumes (Chavan *et al.*, 1986) and it further increased significantly by 13.63% ($p < 0.01$) due to elevated atmospheric CO₂ exposure. Thus, our result of improvement in gelation capacity of chickpea flour under elevated CO₂ may be attributed to the significant increase in starch (20.3%) and reducing sugar content (27.6%) as mediated by atmospheric CO₂ enrichment. The increasing amount of carbohydrates was reported to decrease the thermodynamic affinity of proteins for aqueous solution and magnifies the magnitude of interaction between the protein molecules, thus improving the gelling capacity (Adebawale and Adebawale, 2008).

8.5 Conclusions

Elevated CO₂ has its least influence on individual seed size and dimension but has a major influence on hydration properties. However, seed germination was negatively affected by atmospheric CO₂ enrichment, with reduced activity of the enzymes related to seed germination and seed viability. It may be due to increase in seed coat permeability and reduction in membrane integrity with reduced seedling vigor, although the seedling growth was least affected. Although elevated CO₂ increased seed reserve of carbohydrates but the effect is diminished due to reduction in seed protein content and amino acid concentration for chickpea. Other than N content, nutrient dilution effect of elevated CO₂ was also prominent for K, Ca, S and Zn with increased seed C content that might result in poor nutritional supply for the seedling under elevated CO₂ condition. Prominent reductions in Ca: P ratio also implies the

possibility for nutritional imbalance for grains developed under elevated CO₂. Atmospheric CO₂ enrichment made the grains more carbonaceous with reduced grain protein and oil content, along with reduced K, Ca, S and Zn content. Although elevated CO₂ was seen to cause its least influence on flour physical characteristics (likely, pH and bulk density) and some of the functional characteristics (foaming and cooking properties), its negative effect on flour water and oil absorption characteristics with increased gelation characteristics was, undesirable. However, decreased crude fibre content increases the palatability of chickpea flour along with reduced total free amino acid content and signifies positive impact of elevated CO₂ on chickpea flour quality.

Table 8.1a: Physical attributes of chickpea seeds as affected by atmospheric CO₂ enrichment

		Seed weight (g/100 seeds)	Seed volume (ml/100 seeds)	Seed density (g/ml)	Seed Moisture %	Hydration capacity/seed	Hydration index	Swelling capacity /seed	Swelling index
2010-11	Elevated CO ₂	26.50	18.40	1.44	7.46	0.22	0.83	0.22	1.26
	Ambient CO ₂	25.07	17.93	1.45	7.07	0.18	0.71	0.18	1.02
	p value	0.38	0.21	0.44	0.11	0.01	0.02	0.04	0.02
2011-12	Elevated CO ₂	23.28	16.43	1.41	7.55	0.21	1.00	0.23	1.41
	Ambient CO ₂	21.59	15.93	1.35	6.92	0.19	0.84	0.17	1.06
	p value	0.02	0.28	0.06	0.08	0.03	0.01	0.01	0.01

Table 8.2: Seed germination and seedling vigour of chickpea as affected by elevated atmospheric CO₂

		Germination %	Speed of Germination	Vigour index I	Vigour index II	Root length (cm)	Shoot length (cm)	Total length of seedling (cm)	Weight of seedling (g)	Seed leachate Conductivity (mS ⁻¹ cm ⁻¹ 25 seeds)
2010-11	Elevated CO ₂	64.500	22.185	573.983	3.288	9.025	9.285	9.155	0.053	1.285
	Ambient CO ₂	89.500	35.657	779.971	4.927	8.600	8.885	8.742	0.057	1.166
	p value	0.002	0.002	0.003	0.002	0.431	0.512	0.423	0.578	0.038
2011-12	Elevated CO ₂	65.500	21.167	635.794	3.666	8.207	8.271	9.778	0.046	1.471
	Ambient CO ₂	87.000	35.057	792.936	4.718	7.472	7.897	9.141	0.046	1.255
	p value	0.003	0.008	0.007	0.011	0.206	0.569	0.198	0.851	0.016

Table 8.3a: Changes in macronutrient composition of chickpea grains under elevated CO₂

		C %	N %	C:N	K (mg/g)	P (mg/g)	Ca (mg/ g)	Ca: P	Mg (mg/g)	S (mg/g)
2010-11	Elevated CO ₂	49.982	3.038	16.459	17.163	2.987	1.519	0.509	0.906	1.376
	Ambient CO ₂	43.705	3.383	12.916	25.609	2.849	2.059	0.724	0.918	1.941
	p value	0.001	0.002	0.005	0.0032	0.298	0.002	0.007	0.106	0.003
2011-12	Elevated CO ₂	52.515	3.181	16.504	21.623	3.882	1.967	0.506	0.701	1.415
	Ambient CO ₂	44.079	3.476	12.678	31.139	3.963	2.737	0.689	0.716	2.076
	p value	0.001	0.0002	0.012	0.013	0.107	0.008	0.009	0.905	0.003

Table 8.3b: Changes in micronutrient composition of chickpea grains under elevated CO₂

		Cu (µg/g)	Zn (µg/g)	Fe (µg/g)	Mn (µg/g)	B (µg/g)
2010-11	Elevated CO ₂	1.733	19.308	27.497	25.857	18.151
	Ambient CO ₂	1.513	32.756	28.483	27.132	24.848
	p value	0.216	0.005	0.293	0.669	0.104
2011-12	Elevated CO ₂	1.569	25.534	26.646	28.891	23.244
	Ambient CO ₂	2.283	39.919	29.369	27.742	16.242
	p value	0.085	0.023	0.086	0.713	0.178

Table 8.4: Characteristics of chickpea flour as influenced by elevated CO₂

		Physical properties		Functional properties					
		Bulk Density (g/ml)	pH	Cooking time (min)	Water uptake ratio	Oil absorption capacity (g/g)	Water absorption capacity (g/g)	Foaming capacity (%)	Foam stability (%)
2010-11	Elevated CO ₂	0.516	6.14	57.2	2.035	0.930	1.716	36.318	17.341
	Ambient CO ₂	0.491	5.94	58.1	1.987	1.235	1.126	32.672	15.672
	p value	0.274	0.143	0.9	0.594	0.026	0.0130	0.034	0.0252
2011-12	Elevated CO ₂	0.513	6.02	54.9	1.881	0.946	1.667	37.368	19.671
	Ambient CO ₂	0.504	6.0	53.3	1.873	1.355	1.233	34.676	18.655
	p value	0.852	0.856	0.7	0.873	0.002	0.013	0.099	0.0520

Table 8.5: Least gelation concentration (LGC) of chickpea flour after heating in boiling water for 1h followed by cooling for 2 h at 25±1°C

	Elevated CO ₂							
	2011				2012			
Concentration (%)	R ₁	R ₂	R ₃	R ₄	R ₁	R ₂	R ₃	R ₄
2	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-
8	-	-	-	-	gel	-	-	-
10	gel	gel	gel	gel	firm gel	gel	gel	gel
12	firm gel	firm gel	firm gel	firm gel	firm gel	firm gel	firm gel	firm gel
14	firm gel	firm gel	firm gel	firm gel	firm gel	firm gel	firm gel	firm gel
16	very firm gel	firm gel	firm gel	firm gel	firm gel	firm gel	firm gel	firm gel
18	very firm gel	very firm gel	firm gel	firm gel	very firm gel	very firm gel	very firm gel	very firm gel
20	very firm gel	very firm gel	very firm gel	very firm gel	very firm gel	very firm gel	very firm gel	very firm gel
	Ambient CO ₂							
	R ₁	R ₂	R ₃	R ₄	R ₁	R ₂	R ₃	R ₄
2	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-

6	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-
14	-	gel	gel	gel	gel	gel	-	gel
16	gel	firm gel	firm gel	firm gel	firm gel	firm gel	gel	firm gel
18	firm gel	firm gel	firm gel	firm gel	firm gel	firm gel	firm gel	firm gel
20	firm gel	firm gel	firm gel	firm gel	firm gel	very firm gel	firm gel	firm gel

* $R_x \Rightarrow$ Number of replications (x)

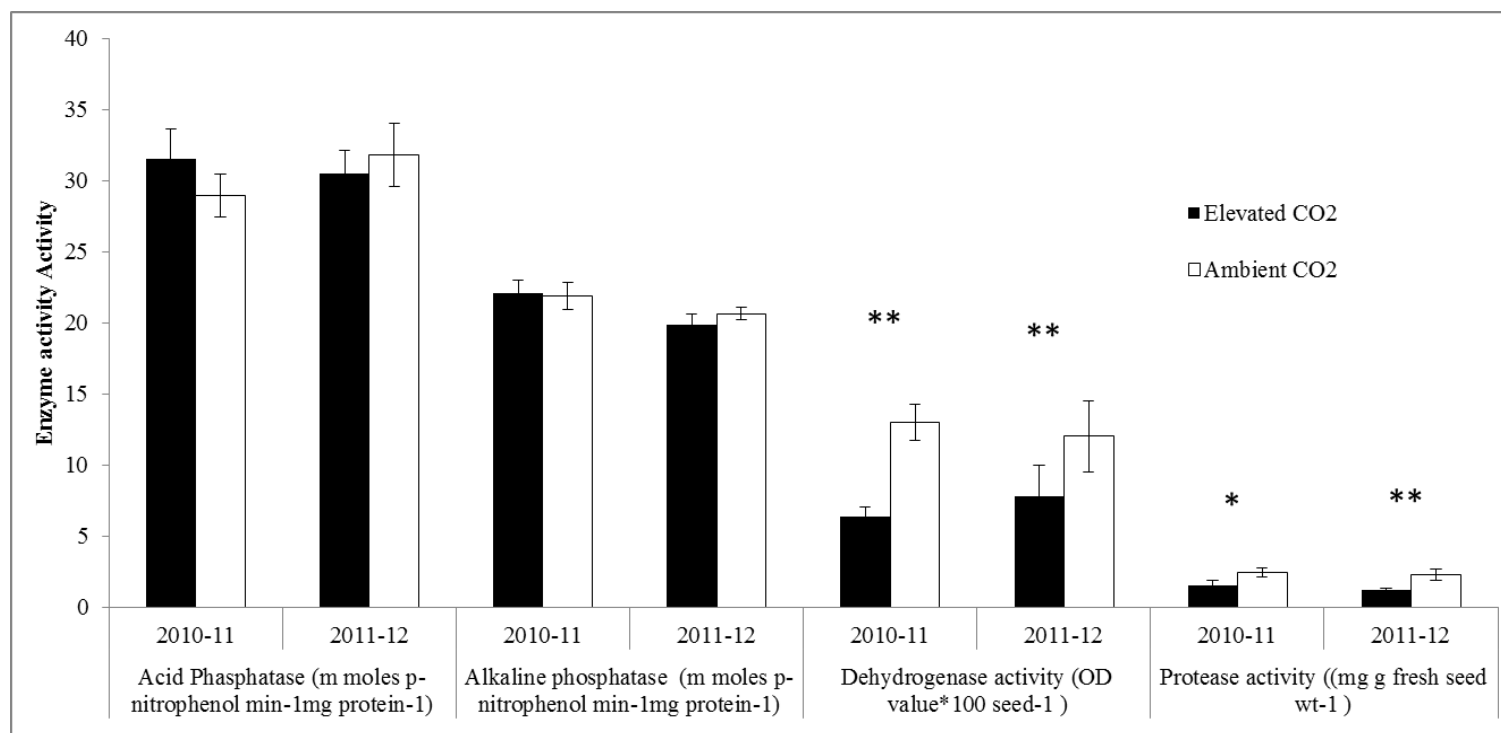


Figure 8.1: Changes in enzyme activity in chickpea seeds grown under elevated and ambient CO₂ treatments

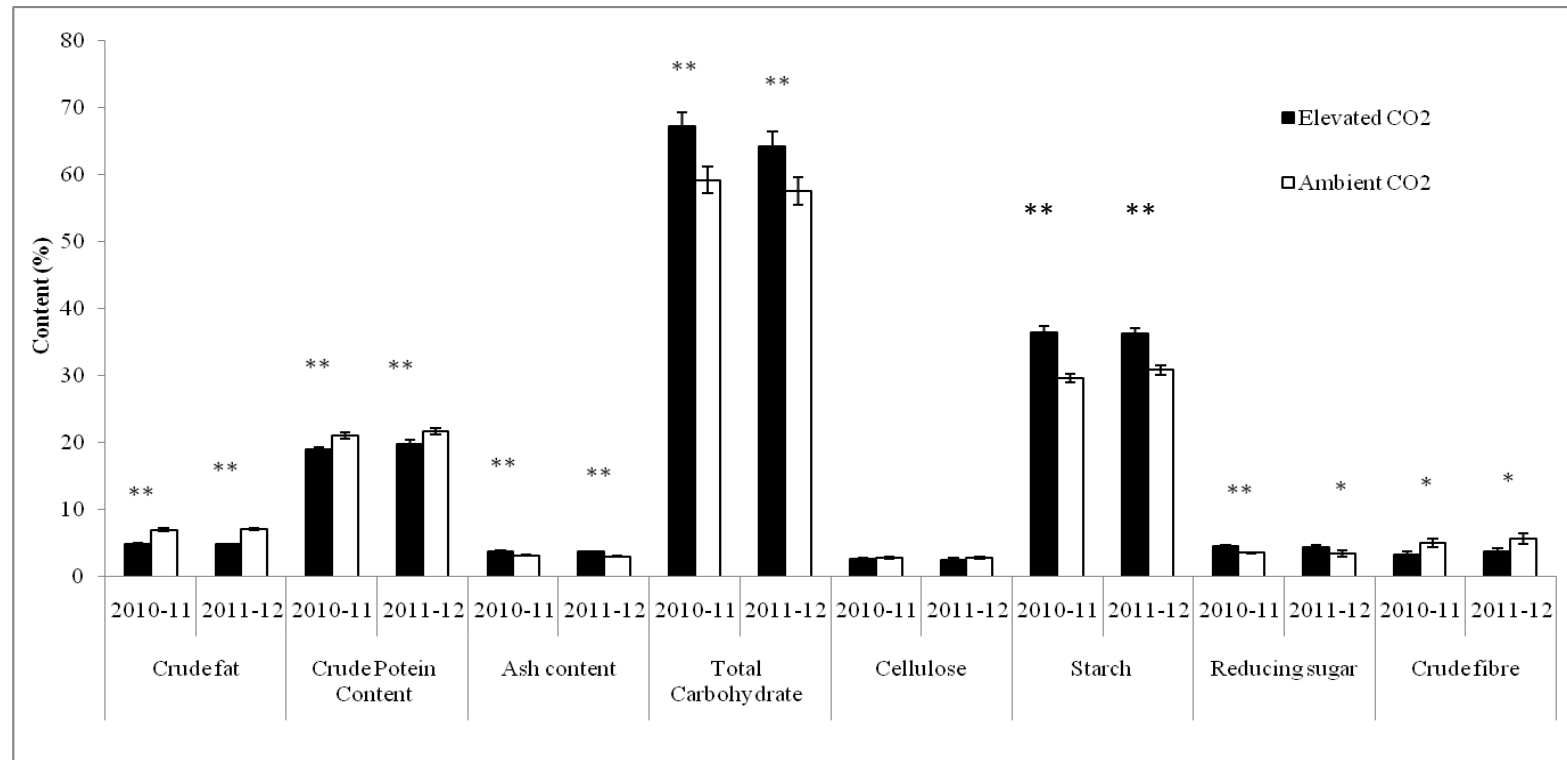


Figure 8.2: (a) Compositional changes in biological macromolecules in chickpea seeds grown under elevated and ambient CO₂ treatments

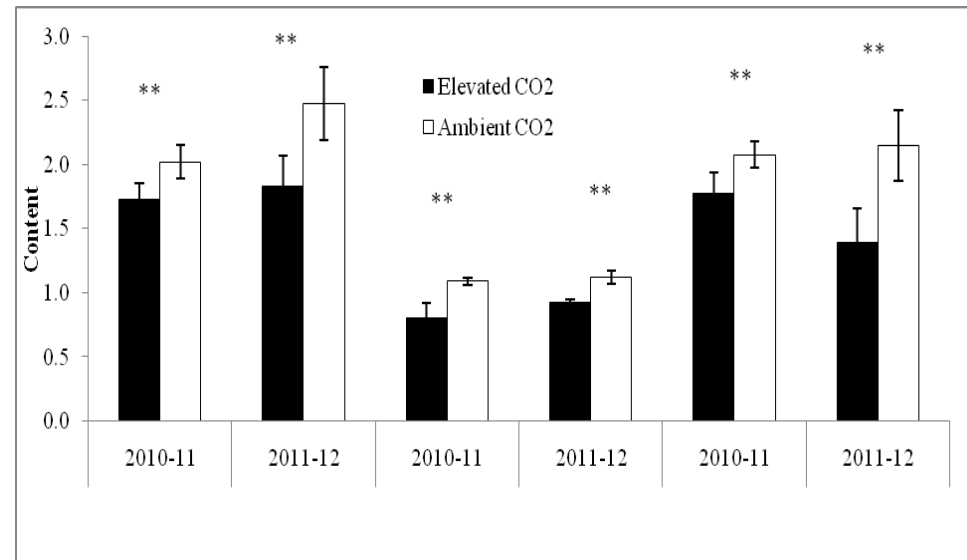


Figure 8.2: (b) Compositional changes in phenol, proline and total free amino acid content in chickpea seeds grown under elevated and ambient CO₂ treatments

9. GENERAL DISCUSSION

The rise in atmospheric CO₂ concentration is one of the best documented global atmospheric changes of the past half century (Prentice, 2001) and is expected to be nearly double during this century (Long *et al.*, 2004). The consequent increase in global temperature has led to considerable interest in the potential of biological systems to mitigate the effects of rising atmospheric CO₂ concentrations by enhanced C sequestration. Enormous research efforts have been undertaken to understand how plants under both natural and managed ecosystems, will respond to rising CO₂.

Chickpea, a C₃ legume is widely grown under rainfed condition. Because CO₂ is a substrate for photosynthesis, an increase in its concentration stimulates photosynthetic assimilation rates in this legume (Kimball *et al.*, 2002). This is documented in increase in leaf-soluble carbohydrates under elevated CO₂ of the present OTC study. However, this response might be cultivar specific and depends on the prevalent environmental conditions (Jablonski *et al.*, 2002) and soil nutrient availability, mostly nitrogen (Kim *et al.*, 2001).

During the two years of experiment, maximum and minimum air temperatures varied by 3-4°C and 1-2°C, respectively, inside the OTC, but no change in soil temperature was detected. The primary effects of rising CO₂ on chickpea plants are in agreement with literature available for other C₃ pulses like *Lolium perenne* (Daepp *et al.*, 2001) pigeon pea (Saha *et al.*, 2012) and soybean (Oikawa *et al.*, 2013). Increase in plant height under elevated CO₂ was accompanied with a prominent increase in LAI. The effect was more prominent at 50% flowering and active pod filling stages. Elevated CO₂ accelerated plant height by 45% on 94 DAS during 2010-11 and 13% on 72 DAS during 2011-12, with a significant increase in LAI ($p < 0.05$). The growth enhancement in chickpea may be interceded through higher nutrient uptake by the plants with increased photosynthetic assimilation process (Bhatt *et al.*, 2007). Increased canopy photosynthesis enables plants to utilize more substrates for growth with concomitant increase in LAI, resulting from enhanced N uptake. As chickpea is a leguminous crop with N-fixing nodules, the possibility of increased N-fixation under elevated CO₂ can be utilized to support the growth enhancement (Gamper *et al.*, 2005). The CO₂ fertilization effect in augmenting the LAI has been widely

documented (Kim *et al.*, 2001; Dermody *et al.*, 2008) with increased photosynthesis rates. Terrestrial plants with the C₃ photosynthetic pathway respond to short-term elevated CO₂ exposure via increased net photosynthesis, by increasing the carboxylation rate of Rubisco and competitively inhibiting the oxygenation of Ribulose-1, 5-bisphosphate (RuBP) (Drake *et al.*, 1997).

Decrease in canopy extinction coefficient signifies that elevated CO₂ enables the plant to modify its canopy architecture towards more erectness. Although LAI amplification was prominent, reduction in canopy extinction coefficient due to canopy erectness (Smart *et al.*, 1994; Brooks *et al.*, 2000) might explain no significant change in fIPAR values during the entire growth period (De Costa *et al.*, 2006; Manderscheid *et al.*, 2009). As a result, atmospheric CO₂ enrichment caused increase in the crop radiation use efficiency (Clifford *et al.*, 1993; Manderscheid *et al.*, 2009), with better utilization of solar radiation (Mulholland *et al.*, 1998) and higher photosynthetic assimilation rate (Walcroft *et al.*, 2005). This enabled the plants to improve the efficiency to augment dry matter accumulation (Drake *et al.*, 1997) for higher produce.

Increased canopy IR temperature (De Costa *et al.*, 2003; Triggs *et al.*, 2004), mediated by increase in stomatal resistance (Morgan *et al.*, 2004; Robredo *et al.*, 2007) and leaf water potential (Wullschlegel *et al.*, 2002b; Nelson *et al.*, 2004) under atmospheric CO₂ enrichment signifies reduced root water uptake. Our result is supported by previous reports on wheat (Hunsaker *et al.*, 2000), maize (Chun *et al.*, 2011), potato (Magliulo *et al.*, 2003), sorghum (Conley *et al.*, 2001) and soybean (Bernacchi *et al.*, 2007). However, higher rate of photosynthesis improved the water-use efficiency through increase in biomass and yield under elevated CO₂ (Ge *et al.*, 2011; Saha *et al.*, 2011). The increase in leaf water content is associated with increase in stomatal resistance. The CO₂-induced stomatal closure may result in increased leaf water potential due to the changes in plant structure, *i.e.*, vessel or tracheid anatomy and leaf specific conductivity (Tyree and Alexander, 1993). Significantly higher Normalized Difference Water Index (NDWI) in both the years suggests that canopy exposed to atmospheric CO₂ enrichment bears more water in the leaf tissue (Gao *et al.*, 1996). NDWI is also an indirect estimation of canopy condition, due to its close association with Normalized Difference Vegetation Index (NDVI, Chen *et al.*, 2005).

Thus, increasing trend of NDWI is also an indirect indicator of better plant growth and vigour with higher level of photosynthetic activity (Peters *et al.*, 2002) under elevated CO₂. Thus, plants may consume either more or less water than normal, but utilize both water and radiation to sufficiently higher levels at elevated than that at ambient CO₂ (Hui *et al.*, 2001). Elevated CO₂ fertilization plays a beneficial role for chickpea by enhancing its harvest index (Vanaja *et al.*, 2008) with increased seed yield factor (Ainsworth *et al.*, 2002; Twine *et al.*, 2013).

Significant increase in leaf chlorophyll content (Fig. 5a) along with decrease in leaf carotenoids content (Fig. 5b) under elevated CO₂ over the entire crop growth period implies elevated CO₂ induces protection against leaf chlorophyll damage, and thus ensuring the greenness of the leaves for a longer duration (Donnelly *et al.*, 2000). Increase in leaf chlorophyll content may be an indirect indicator of more N utilization under elevated CO₂, but decrease in leaf carotenoids strongly indicates an increasing shelf-life of leaf chlorophyll in chickpea with enhanced photosynthesis under elevated CO₂ environment. Wang *et al.* (2004) reported that elevated CO₂ increased chloroplast number per unit cell area. Being a legume, chickpea can satisfy its N requirement through symbiotic fixation. Our results indicate that under no limitation in available N supply, N uptake by plants can be accelerated under elevated CO₂ resulting in an increase in the foliar N concentration and leaf chlorophyll content (Cheng *et al.*, 2010). Increasing (although non-significant) stem dry matter allocation was recorded under elevated CO₂, signifying the enhanced downward movement of photosynthates, due to increased photosynthetic assimilation under elevated CO₂. Thus, significant increase in chickpea growth and higher dry matter allocation towards the stem under elevated CO₂ could be attributed to photosynthetic enhancement effect of CO₂ and partitioning of photosynthates to aboveground plant parts (Pal *et al.*, 2004). The extra C fixed by the plants was translocated to growing axis of the plant species (Sharma and Sengupta, 1990).

Furthermore, increase in specific leaf N (SLN) at vegetative and 50% flowering stage is also an important determinant for increased radiation-use-efficiency in chickpea (Brodrick *et al.*, 2013) controlling the leaf photosynthetic responses (Sinclair *et al.*, 2000; Milroy and Bange, 2003). Reduction in SLN during maturity of pods is associated with seed filling (Hall *et al.*, 1995) due to greater mobilization of

leaf N towards growing pods under elevated CO₂ environment (Gifford *et al.*, 2000; Pal *et al.*, 2004). Plant's alteration in N uptake and utilization under elevated CO₂ environment has been reported (Leakey *et al.*, 2009), possibly due to excess available C bringing a change in N composition and partitioning, modifying the leaf C:N ratio (Rogers *et al.*, 2004; Ainsworth *et al.*, 2006). Reduction in water-soluble carbohydrates at pod maturity (Miller *et al.*, 1997) and a wider C:N ratio under elevated CO₂ implies photosynthetic acclimation (Ainsworth and Long, 2005). This acclimation may accelerate the senescence by producing systemic signals to increase the N availability by releasing it from old leaves (De la Mata *et al.*, 2012). The stimulation in root growth in chickpea is anticipated from rising atmospheric CO₂ with enhanced dry matter allocation towards the roots (Bhattacharyya *et al.*, 2013) and improvement of nodulation properties (Serraj *et al.*, 1998; Lee *et al.*, 2003) due to enhanced microbial biomass (Das *et al.*, 2011) in the soil. Root length and surface area densities appear to specifically increase through CO₂ fertilization, as compared to other root parameters. Results suggest an increased dry matter allocation to roots, which is supported by previous studies (van Ginkel *et al.*, 2000; Gai-ping *et al.*, 2006; Austin *et al.*, 2009; Bhattacharyya *et al.*, 2013). There was positive response in soil microbial biomass C (1.6 to 2.7 times higher under elevated CO₂). Increase in microbial biomass C under elevated CO₂ has also been reported by Li *et al.* (2004b), Das *et al.* (2011) and Bhattacharyya *et al.* (2013).

No general consensus on the change in soil C through CO₂ fertilization could be observed, although increase in top-soil C in diverse temperate ecosystems has been reported (Jastrow *et al.*, 2005). Total soil C and N increased marginally (along with marginal increase in C:N ratio) although the changes were non-significant. Wider C:N ratio indicates more (biologically) active rhizosphere, as argued by Anderson *et al.* (2011). Except labile C (12N in modified Walkley-Black and MBC), all other C pools including Scholtenberger-C declined under elevated CO₂. Results suggest an increased microbial response under CO₂ fertilization (De Graaff *et al.*, 2006, Carney *et al.*, 2007; Inubushi *et al.*, 2011; Saha *et al.*, 2011). Furthermore, greater root growth results in augmented C inputs (Lukac *et al.*, 2003; Godbold *et al.*, 2006) and thus increasing the active labile C fraction in the soil (Hoosbeek *et al.*, 2006; Kant *et al.*, 2007; Ross *et al.*, 2013). Dominating impact of elevated CO₂ on soil microbial

activities and their controls over ecosystem N dynamics can be mediated through CO₂ stimulation of symbiotic N₂ fixation (Rogers *et al.*, 2006; Hu *et al.*, 2006). Total soil carbohydrate C was less than their initial values, the decrease was more under elevated CO₂. This explains higher decomposition of organic substrate by microbes under elevated CO₂. While, water-soluble carbohydrate C reduced under elevated CO₂, it showed a net increase under ambient CO₂. This apparent contrasting result suggests still higher activity of microbes in utilizing the residual (labile) water-soluble carbohydrate C under the elevated CO₂. Higher Soil CO₂ flux indicates greater microbial respiration. Our results are in agreement with Moore *et al.* (2008), but in contrast to no change in soil and root respiration result by Fitter *et al.* (1996). Significant positive relations between root biomass and root respiration have been reported (Pregitzer *et al.*, 2000). The increase in microbial respiration is closely related to greater amount of root exudation and above-ground litter (Zak *et al.*, 2000). Greater microbial respiration in our study corroborates with greater root biomass, higher litter inputs under elevated compared to ambient-CO₂ treatments (Bernhardt *et al.*, 2006; George *et al.*, 2003).

A significant increase in acid and alkaline phosphatase activities under elevated CO₂ was observed, as also reported by others (Lagomarsino *et al.*, 2008; Das *et al.*, 2011; Manna *et al.*, 2013). Alkaline phosphatase was derived from microorganisms only, while acid phosphatases are contributed to roots with associated mycorrhiza and other fungi (Tarafdar and Marschner, 1994). Evidence was also available on plant roots producing phosphatase enzyme capable of dephosphorylating the organic P (Greaves and Weblay 1965). Alkaline phosphatase activity is more than the acid phosphatase as the soil used in the present study was slightly alkaline (Das *et al.*, 2011). Chickpea is reported to exude progressively higher amounts of organic acids in soil that increases the availability of phosphorus to the plant (Veneklaas *et al.*, 2003). Our soil was deficient in available P, and elevated CO₂ brought greater rhizosphere acidification, exudation of organic anions and secretion of phosphatase from plant roots in response to P deficiency (Hinsinger *et al.*, 2005; Richardson *et al.*, 2005). This elucidates positive impact of CO₂ fertilization on mobilizing P from soil P reserve (insoluble), especially in P-deficient soils. Increase in soil dehydrogenase and fluorescein diacetate activities under elevated CO₂ is supported by other studies (Das *et al.*, 2011; Bhattacharyya *et al.*, 2013), although no change has been reported in rice

(Manna *et al.*, 2013). Higher atmospheric CO₂ increases the allocation of photosynthates to roots resulting in increased root growth and rhizodeposition (Haase *et al.*, 2007; Anderson *et al.*, 2011) and microbial respiration (De Graaff *et al.*, 2006). Increase in enzyme activities promote better nutrient availability in soil (Jin and Evans, 2007) and also increase in number and activities of microbes (Dhillon *et al.*, 1996). Since microbes use the translocated photosynthates as C and energy source (leading to higher activities and build up of biomass), increase in labile C under elevated CO₂ validates the hypothesis of higher exudation of photosynthates from the roots leading to proliferation of microbes. Greater microbial activities can reduce soil C stock by bringing recalcitrant pools to labile fractions, which is subsequently lost as CO₂ flux. This may act as the counterbalance to increased soil C addition (litter and rhizodeposition) under CO₂ enrichment. Therefore, the common notion of higher C build-up in soils through CO₂ enrichment may not be universally true. Elevated CO₂ may constrain the potential for net gains in soil C suggesting a limited capacity of earth's ecosystems to stabilize atmospheric CO₂ and slow global warming (Carney *et al.*, 2007). Role as sink for long-term C storage under increased atmospheric CO₂ has been questioned in forest soils also (Schlesinger and Lichter, 2001).

Elevated CO₂ not only increases the number and biomass of nodules (Schortemeyer *et al.*, 2002), it brings change in biological macromolecular composition, mediated by improved C supply (Morgan *et al.*, 2005a; Rogers *et al.*, 2006) with higher soluble sugar and starch accumulation in the nodules (Cabrerizo *et al.*, 2001; Aranjuelo *et al.*, 2009b). The increase in nitrogenase activity (Temperton *et al.*, 2003a) and the leghaemoglobin content (Haase *et al.*, 2007) also indicates increased N₂ fixation capacity of chickpea nodules, due to close coupling between C assimilation and symbiotic N₂ fixation (Schortemeyer *et al.*, 2002; Gamper *et al.*, 2005). This induces chickpea to fix more N, as evidenced by greater nodule N (Rogers *et al.*, 2006), and thereby stabilizing C:N ratio, when exposed to atmospheric CO₂ enrichment (West *et al.*, 2005). Moreover, increase in soil microbial activity signifies amplification in all soil enzyme activities due to elevated CO₂ exposure. This has a significant role in increased soil C mineralization and therefore, greater soil CO₂ flux and nutrient mobilization process due to rhizosphere acidification. In this process, a larger rhizodeposition occurs under elevated CO₂ condition inducing greater nutrient availability for higher crop growth and yield.

Although effect of atmospheric CO₂ increase is prominent in many aspects of growth and development of chickpea, its effect on produce (grain) quality remains fuzzy. In accordance to subsequent increase in hydration and swelling indices, reduced seed germination percentage was noted under elevated CO₂ (Quaderi and Reid, 2005) with subsequent reduction in seed viability, protease activity and increased seed leachate conductivity. Increased C content with decrease in N in seeds resulted in widening of C:N ratio (Jablonski *et al.*, 2002), with subsequent dilution of nutrients likely K, Ca, S and Zn (Högy and Fangmeier, 2009b; Högy *et al.*, 2013). No changes in flour quality like flour pH, bulk density and foaming characteristics was observed. Seed gelation property improved with reduction in seed protein content and increased carbohydrate fractions (Picon-Cochard *et al.*, 2004). Reduction in phenol and crude fibre content was indicative of the positive effect of CO₂ enrichment due to increase in the protein digestibility and palatability of the flour from nutritional viewpoint. However, increase in other carbohydrate fractions (Donnelly *et al.*, 2001) and reduction in crude protein content (Taub *et al.*, 2008) may offset this effect. Increase in water capacity of chickpea flour with a subsequent reduction in oil absorption capacity indicates the tendency of elevated CO₂ to make the flour more carbonaceous with reduced grain quality (Blumenthal *et al.* 1996).

10. SUMMARY AND CONCLUSIONS

Human activities are causing rapid changes in the atmosphere and climate that directly impact agriculture production. Atmospheric and climate change began accelerating after the industrial revolution. CO₂ concentrations which averaged about 270 ppm prior to the industrial revolution, have now surpassed 380 ppm, and will exceed 550 ppm by 2050 (Long *et al.*, 2004). Plant photosynthetic processes and consequently plant growth are known to be directly affected by elevated CO₂ (Kimball *et al.*, 2002).

Determining whether terrestrial ecosystems will buffer or exacerbate increasing atmospheric carbon dioxide concentrations over the next century requires knowledge of long term carbon storage in soil and vegetation. In the last decade, a growing concern about the potential consequences of climate change on soil processes, coupled with a desire to develop methods of improving carbon sequestration, have stimulated experimental research, modeling and theorizing (Zak *et al.*, 2011). Carbon in soil affects the formation and stabilization of aggregates which is an important parameter for preventing soil loss through water and wind erosion. Similarly, increased biomass production caused by elevated CO₂ concentration implies more carbon input into the soil which could alter soil physical properties. Therefore, it is important to study the changes in microclimate of the soil and canopy of the plant system brought about by elevated CO₂ and its impact on plant growth, dry matter partitioning, root dynamics, active soil carbon pool and soil physical characteristics in important food crops.

Field experiments were conducted on chickpea (*Cicer aurietinum* L; var *kabuli*) in open top chambers (OTC) at IARI farm to study the effects of elevated CO₂ (580±20 ppm) on radiation use, growth, yield, soil water, root growth, soil C, N and associated biophysical characteristics. Additionally, measurements were taken on the quality of the chickpea produce as influenced by atmospheric CO₂ enrichment. The control treatment had ambient CO₂ concentration of 385 ppm. Salient findings of the study are summarized below:

1. Elevated CO₂ resulted in increase in RUE in chickpea on two accounts. Firstly the canopy structure become more erect as shown by the decrease in radiation

extinction coefficient, thus leading to better distribution and interception of radiation. Secondly CO₂ fertilization led to significant increase in biomass which also increased RUE.

2. Although total leaf area per plant basis increased under elevated CO₂ but decrease in specific leaf area (SLA) indicated appearance of thicker leaves than that of control. Higher specific leaf Nitrogen (SLN) led to the increased crop RUE under elevated CO₂ with increased soluble carbohydrate production in leaf. Although both C and N content in leaves increased, magnitude of increase was higher in C, which resulted in a wider C:N ratio in leaves at 50% flowering stage. This could possibly accelerate the early leaf senescence under elevated CO₂. But the greater mobility of soluble carbohydrate from leaf towards the economic plant parts suggests the possibility of early senescence of elevated CO₂ grown chickpea plants as was reflected in canopy LAI data also.
3. Profile (0-90 cm) moisture data showed no significant difference between cumulative soil moisture depletion with no significant change in canopy conductance (slope of cumulative water use vs. intercepted PAR) for chickpea plants exposed to elevated and ambient CO₂ treatments. The CO₂ fertilization effect of increasing the leaf area index proves its potentiality to compensate the suppressing impact of elevated CO₂ on leaf transpiration rate (due to increase in leaf diffusive resistance). However, water use efficiency (WUE) was significantly higher under elevated condition due to greater crop biomass and yields.
4. Although leaf water potential and relative water content was higher under elevated CO₂, canopy temperature was higher due to increased diffusive resistance and reduced transpiration.
5. Significant increase in root weight and length densities and surface area in both the 0-15 and 15-30 cm layers was recorded under elevated CO₂. Increase in nodule size, number and specific surface area indicated larger partitioning of photosynthates (total carbon and carbohydrate content in nodules increased significantly) towards below-ground root nodules. The nitrogen fixing

capacity of the plant increased due to increase in nitrogenase activity and legheamoglobin content in nodules; thereby maintaining the C:N ratio.

6. Under elevated CO₂ the rhizospheric soil did not show any variation in soil physical properties like bulk density and saturated hydraulic conductivity, water retention at -33 and -1500 kPa, but significant increase in mean weight diameter under two pre-treatments (mechanical break down and fast wetting) was observed. The labile pools of soil C increased, while recalcitrant C decreased under elevated CO₂.
7. Activity of soil enzymes (β -Glucosidase, dehydrogenase and FDA hydrolysis) increased substantially with atmospheric CO₂ enrichment, which substantiated the role of active C to stimulate soil microbial population. Increase in acid and alkaline phosphatase activities was recorded, which brought the non labile soil phosphorus into the available form for utilization by the plant. Therefore, elevated CO₂ could augment the rhizosphere biological activity, which in-turn helped in greater nutrient availability in the soil.
8. The leaf area index was significantly higher under CO₂ enrichment. Increase in individual plant height, leaf chlorophyll content and dry matter yield, decrease in leaf carotenoids content and specific leaf area suggested the presence of less expanded thick green leaves under elevated CO₂ condition.
9. Due to increase in sink capacity, the seed yield (both the number of mature seeds and pods) increased; though seed test weight remained unchanged under elevated CO₂ exposure. A larger increase in yields as compared to biomass, caused significant increase in harvest index of chickpea.
10. Negative effect of CO₂ on chickpea grain quality was prominent. Seed germination properties were poorer. Moreover, increase in carbohydrate with decreasing grain protein content resulted in significant reduction in oil absorption and increase in water absorption capacity, indicating poor produce quality under elevated CO₂.

Thus, our results suggest that improved soil water-plant relations and radiation utilization, more biologically active rhizosphere including better root growth and

nodulation, enhanced nutrient availability, carbon assimilation in plant produce with wider C:N ratio and reduced nutrient quality in chickpea are some of the possibilities under elevated CO₂ exposure. However, our results indicated a better scope of adoption of chickpea under water limited condition, when CO₂ in the atmosphere is increased. Change in soil C pools can be a significant indicator in deciding C sequestration potential under CO₂ enrichment. These have major consequences for agriculture and native ecosystems in a world with rising atmospheric CO₂. On the other hand, negative effects on seed quality in terms of seed germination characteristics and nutritional imbalance may cause increased C content and dilution of N, K Ca, Zn and S content in chickpea grains.

Future directions:

1. Intensive study was performed to study the effect of atmospheric CO₂ enrichment on crop-soil interaction with the possible changes in their biophysical characteristics for chickpea. Future research effort is essentially needed to intensify the similar search on soil plant atmospheric interaction, but under elevated CO₂ and increased air temperature both in combination. Because elevated temperature is also predicted have a major modifying effect on elevated CO₂ induced crop physiological responses (IPCC, 2007).
2. OTC modifies the crop microenvironment by manipulating the radiation transmission through its plastic cover that also creates barrier to the free wind circulation through the crop canopy, with an increase of 2-4°C in average air temperature from normal outside environment. To study the impact of elevated atmospheric CO₂ on chickpea, free air CO₂ enrichment facility (FACE) will be better than OTCs. There is a need to study the elevated CO₂ induced changes in ecophysiological processes under next generation FACE experiments, as a critical investment to ensure produce quantity and nutritional quality of legumes (chickpea) in the future elevated CO₂ world.
3. The study on changing dynamics of rhizosphere biophysical processes under elevated CO₂ also has its own lacuna. Although total microbial population increased under elevated CO₂, but it is better to study the community based response (dominance of crop-specific groups of bacteria and fungi). There is a

large controversy that whether the elevated CO₂ induced stimulation is crop specific? whether it is confined only to increased microbial population or it also enhances their energy conversion efficiency. This not only controls the plant nutrient uptake but is also a determining factor for rhizosphere C sequestration potential under variable soil environment. In this sense, tracer study may have a good potentiality to study the balance between rhizodeposition and mineralization processes in rhizosphere as altered by elevated CO₂.

4. Varietal specificity is also a major determining factor for the crop responsiveness towards elevated CO₂ environment. Identification and isolation of variety specific positively influenced CO₂ responsive genes and incorporating them into other potential dryland legumes also invades the potentiality of biotechnological interventions to increase the dryland productivity of Indian agriculture (contributing about 55% of total crop production and 48% of total cultivated area).
5. Although a clear indication for reduction in produce quality was prominent, but more in depth rigorous analysis of proximate composition is needed to know the effect of elevated CO₂ on different amino acid composition, fatty acid profiles, vitamins and anti nutritional factors and other associated quality parameters of chickpea grains.
6. To extend the results of two years study a long-term study under varying levels of CO₂ is further needed for actual site specific validation of the obtained results and to model the real effect of climate change on soil plant interaction process by using dynamic crop simulation models.

Effect of elevated atmospheric CO₂ on chickpea (*Cicer arietinum* L.) water use and rhizosphere carbon dynamics

Abstract

A field study was conducted to investigate the impact of elevated CO₂ (580 ± 20 µmol mol⁻¹) on radiation interception, its use, other related physiological parameters, yield components, soil temperature, soil moisture depletion and its use, root characters, soil physical parameters and different fractions of active carbon for *kabuli* chickpea in Open Top Chambers (OTCs) and were compared with those grown under ambient levels of CO₂ (approx. 385 µmol mol⁻¹) in similar OTCs, i.e. control. Though the cumulative radiation interception did not vary between the CO₂ treatments, the radiation extinction coefficient (k) value was lower for plants exposed to elevated CO₂ which indicated a more erect structure for these plants. However, the radiation use efficiency (RUE) was higher by 135.3% for elevated CO₂ grown plants than control. LAI was significantly higher during most of the growth stages and canopy temperatures were lower for plants exposed to higher CO₂ compared to control. On per plant basis, seed yield increased by 82% due to elevated CO₂ exposure as the pod number increased significantly though seed test weight was unchanged. Even though elevated CO₂ resulted in increase in biomass, the proportionate increase in seed yield was higher than the proportionate increase in biomass causing increase in harvest index. Decrease in specific leaf area indicated thicker leaves as unit leaf area also increased with wider leaf C:N ratio due to higher C assimilation in leaf under elevated CO₂.

Chickpea plants grown under elevated CO₂ showed higher stomatal resistance and hence less transpiration and higher canopy temperature. Soil moisture depletion from different profiles showed a trend of lower moisture utilization under elevated CO₂ as compared to ambient CO₂. However, the water use efficiency (WUE) was higher for elevated CO₂ plants as compared to control. Root characters studied at three growth stages showed a significant increase in root weight density, root length density and root surface area in the 0-15 cm soil for plants grown under elevated CO₂. Increased nodulation with higher nitrogen fixing capacity (higher nitrogenase activity and leghemoglobin content) of chickpea nodule enabled plants to increase below

ground allocation of biological macromolecules with stabilization of nodule C:N ratio. After the completion of the crop season, the rhizosphere soil did not show any change in the soil physical properties like bulk density, hydraulic conductivity and but mean weight diameter increased under elevated CO₂. Different active labile carbon pools (12N WBC and microbial biomass carbon) increased significantly due to CO₂ exposure, with a decreasing trend in other stable recalcitrant soil C pools. Also the soil enzymes increased substantially which indicated more viable rhizosphere due to stimulation of microbial population leading to better biological activity in soil near the root zone under CO₂ enrichment. The grain carbohydrate content also increased at the expense of reduction in grain protein content. Thus, the beneficial effect of elevated CO₂ was confined to increase in aboveground biomass production, reduced crop water use and greater nutrient availability in soil. On the other hand increase in labile fractions of soil C pools and decrease in grain quality accounted for the negative impact of atmospheric CO₂ enrichment on chickpea.

चने (साइसर एराइटीनम) में जल-उपयोग एवं जड़ क्षेत्र कार्बन गतिकी पर प्रवर्धित वातावरणीय CO₂ का प्रभाव

सार

प्रस्तुत अध्ययन खुले शीर्ष वाले कक्षों (ओ टी सी) में काबुली चने हेतु, प्रवर्धित CO₂ (580±20 माइक्रो मोल प्रति मोल) का विकिरण अपरोधन, इसके उपयोग, अन्य संबंधित कार्याकीय प्राचलों, उपज घटकों तथा सक्रिय कार्बन के विभिन्न घटकों पर प्रभाव ज्ञात करने के लिए किया गया, साथ ही साथ ओ टी सी में CO₂ के परिवेशी स्तरों (लगभग 385 माइक्रो मोल प्रतिमोल) के अन्तर्गत उगाए गए काबुली चने अर्थात कंट्रोल से तुलना की गई। यद्यपि CO₂ उपचारों के बीच संचयी विकिरण अपरोधन में ज्यादा भिन्नता नहीं थी, किन्तु प्रवर्धित CO₂ के अन्तर्गत रखे गए पौधों हेतु विकिरणविलुप्ति गुणांक (k) मान कम रहे जो उन पौधों की अधिक सीधी संरचना को दर्शाता है। वैसे कंट्रोल की तुलना में, प्रवर्धित CO₂ के अन्तर्गत उगाए गए पौधों हेतु विकिरण उपयोग दक्षता (आर ए यू) 135.3% अधिक पायी गई। कंट्रोल की तुलना में, उच्चतर CO₂ के अन्तर्गत, पौधों की अधिकांश वृद्धि-अवस्थाओं में पत्ती क्षेत्र सूचकांक (एल ए आई) महत्वपूर्ण रूप से अधिक था जबकि वितान-तापमान कम थे। प्रति पौधा के आधार पर, प्रवर्धित CO₂ में उगाए जाने के कारण बीज-उपज में 82% की बढ़ोतरी देखी गई क्योंकि फलियों की संख्या में महत्वपूर्ण रूप से बढ़ोतरी हुई हालाँकि बीज परीक्षण भार में कोई परिवर्तन नहीं हुआ। प्रवर्धित CO₂ के परिणामस्वरूप जैव मात्रा में बढ़ोतरी हुई किन्तु बीज उपज में वर्तमान बढ़ोतरी, जैवमात्रा में वर्तमान बढ़ोतरी की तुलना में अधिक थी जिसके कारण कटाई सूचकांक में बढ़ोतरी हुई। विशिष्ट पर्ण क्षेत्र में कमी मोटी पत्तियों को दर्शाती है क्योंकि प्रवर्धित CO₂ के अन्तर्गत पत्ती में उच्चतर C स्वांगीकरण के कारण अधिक C:N अनुपाते के साथ साथ इकाई पर्ण-क्षेत्र में भी बढ़ोतरी हुई।

प्रवर्धित CO₂ परिस्थितियों के अन्तर्गत उगाए गए चने के पौधों ने उच्चतर स्टोमेटल प्रतिरोधिता दर्शायी, इसलिए कम वाष्पोत्सर्जन हुआ एवं वितान-तापमान अधिक पाया गया। परिवेशी CO₂ की तुलना में, प्रवर्धित CO₂ के अन्तर्गत विभिन्न प्रोफायलों से मृदा-नमी ह्रास ने कम नमी के उपयोग को दर्शाया। वैसे कंट्रोल की तुलना में, प्रवर्धित CO₂ के अन्तर्गत उगाए गए पौधों की जल उपयोग दक्षता (डब्ल्यू यू ई) अधिक पायी गई। फसल की तीन वृद्धि

अवस्थाओं पर अध्ययन किए गए जड़-गुणों ने प्रवर्धित CO₂ के अन्तर्गत उगाए गए पौधों हेतु 0–15 सें.मी. गहराई तक मृदा में, जड़-भार घनत्व, जड़-लम्बाई घनत्व एवं जड़-सतह क्षेत्र में सार्थक रूप से बढ़ोतरी दर्शायी। चने की जड़-ग्रंथियों द्वारा उच्चतर नाइट्रोजन-स्थायीकरण क्षमता के कारण पौधों को जड़ग्रंथि C:N अनुपात के स्थायीकरण सहित जैविक बृहद अणुओं को जमीन के भीतर नियतन में बढ़ोतरी योग्य बनाया। फसल लेने के बाद, मूल-परिवेशी मृदा ने, मृदा के भौतिक गुणों यथा, स्थूल घनत्व, चलजलीय चालकता में किसी प्रकार का परिवर्तन नहीं दर्शाया किन्तु प्रवर्धित CO₂ के अन्तर्गत औसत भार व्यास में बढ़ोतरी देखी गई। CO₂ के प्रवर्धन के कारण विभिन्न सक्रिय परिवर्ती कार्बन पूल्स (12 एन डब्ल्यू बी सी एवं सूक्ष्म जैव जैव पदार्थ कार्बन) में सार्थक बढ़ोतरी हुई जबकि अन्य स्थिर अडिग मृदा कार्बन पूल्स में कमी का क्रम देखा गया। मृदा-एन्जायमों में भी टिकाऊ बढ़ोतरी देखी गई जो सूक्ष्म जीव-आबादी के उद्दीपन के कारण अधिक जीवन क्षम मूल-परिवेश को दर्शाता है जिससे CO₂ प्रवर्धन के अन्तर्गत जड़-क्षेत्र के निकट मृदा में बेहतर जीव विज्ञान संबंधी सक्रियता पायी गयी। दाना-प्रोटीन अंश में कमी होने से दाना-कार्बोहायड्रेट अंश में भी बढ़ोतरी हुई। इस प्रकार, प्रवर्धित CO₂ का लाभकारी प्रभाव, जमीन के ऊपर जैवमात्रा-उत्पादन में बढ़ोतरी, फसल-जल उपयोग में कमी एवं मृदा में पोषक तत्वों की अधिक उपलब्धता थे। दूसरी ओर, मृदा कार्बन पूल्स के परिवर्ती प्रभाजों में बढ़ोतरी तथा दाना-गुणवत्ता में कमी, चने की फसल में, वातावरणीय CO₂ के प्रवर्धन के दुष्प्रभाव थे।

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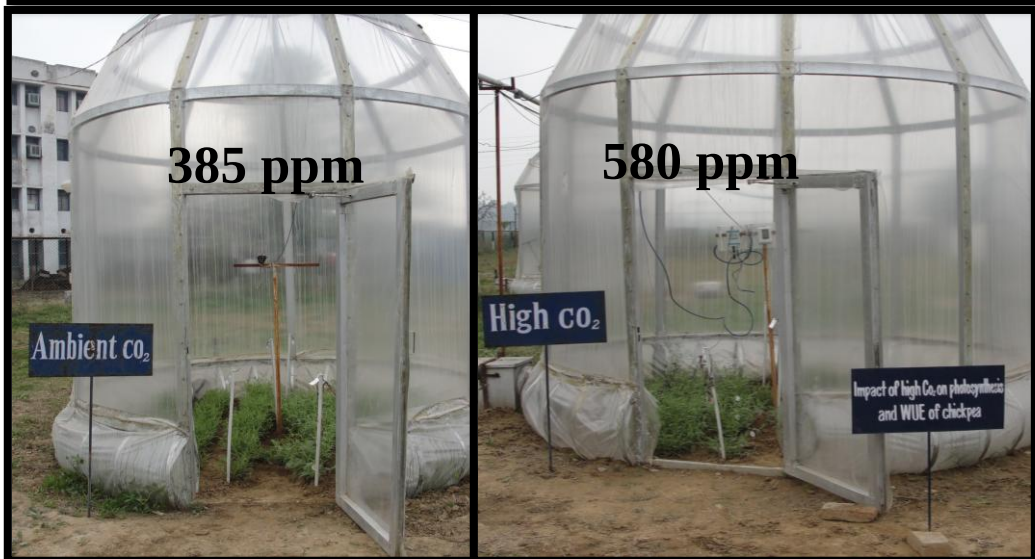
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APPENDIX



Site of Experimentation



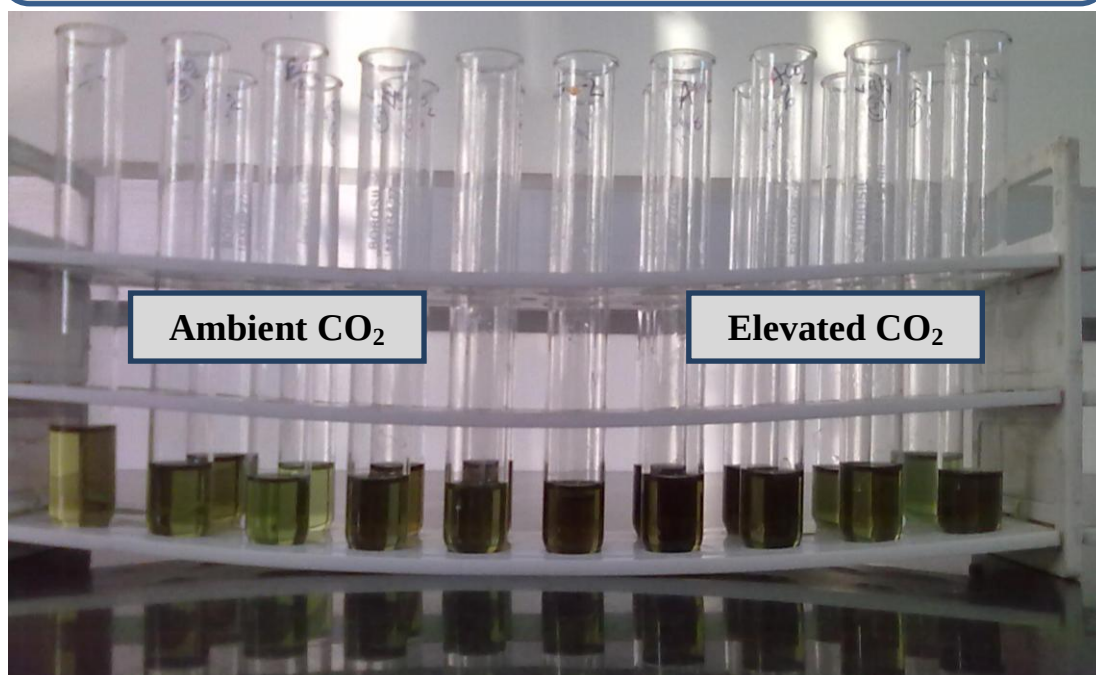
Determination of canopy temperature by IR thermometry



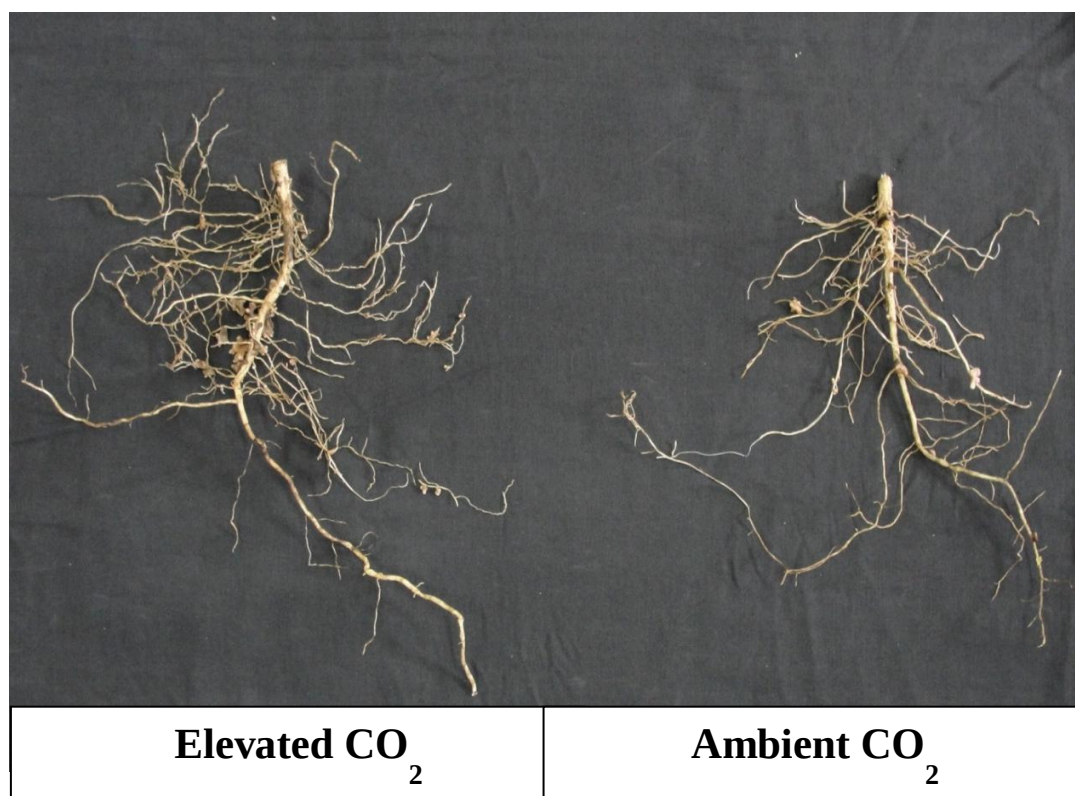
**Determination of crop water use by neutron moisture meter
(Soil moisture depletion method)**



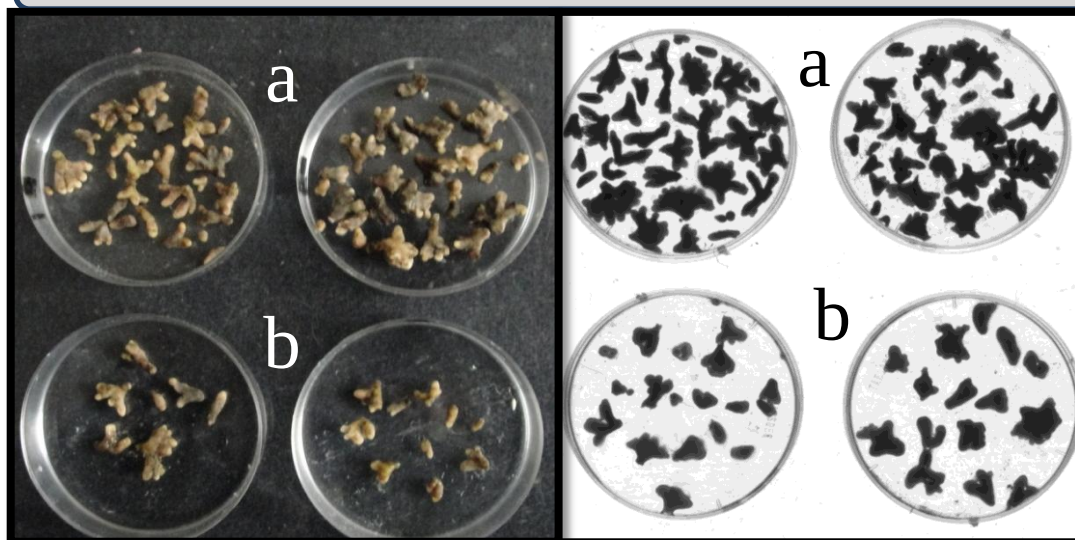
Determination of total organic matter by Scholenberger method



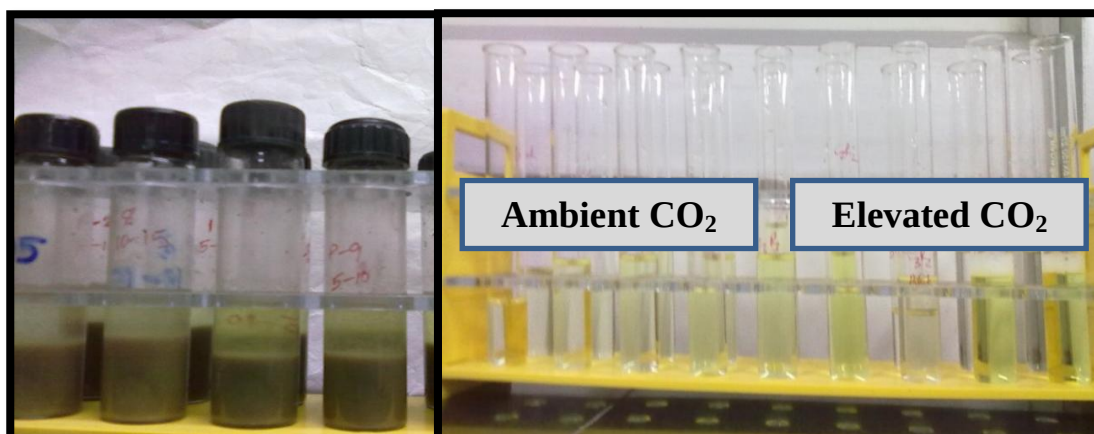
Determination of total carbohydrate carbon in soil by Anthrone method



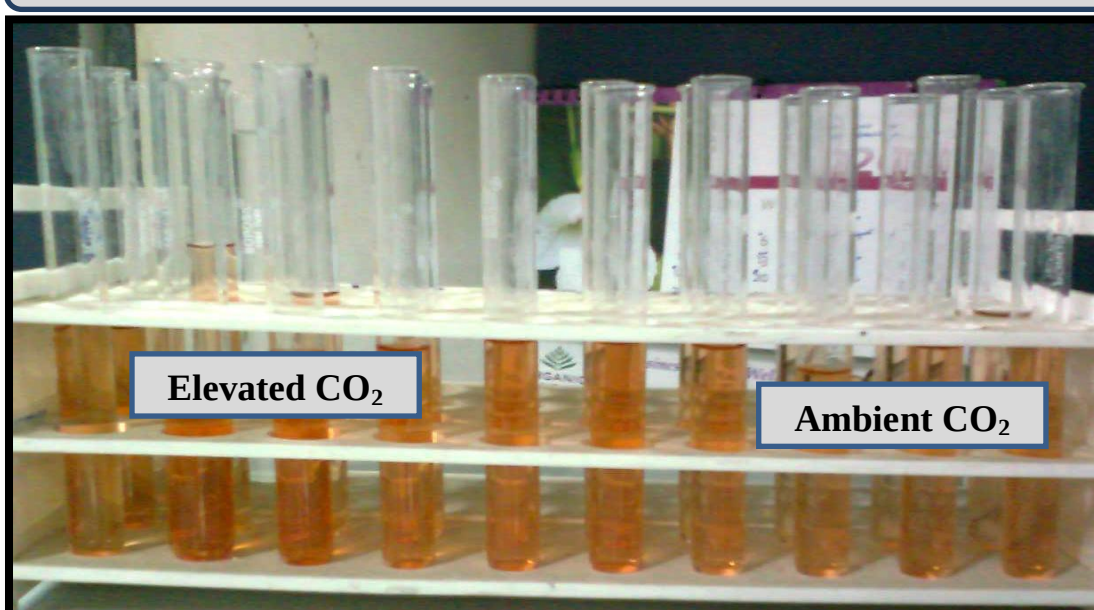
Root growth response of *kabuli* chickpea under elevated CO₂



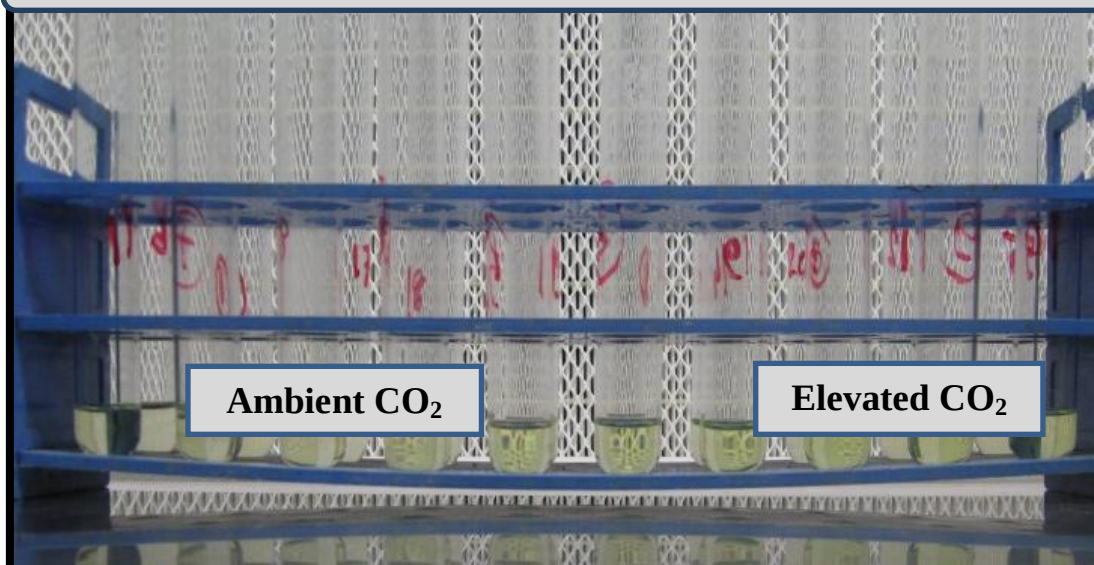
Root nodulation enhancement under (a) elevated and (b) ambient CO₂



Determination of acid phosphatase activity in soil



Determination of dehydrogenase activity in soil



Determination of FDA hydrolysis activity in soil