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Effect of Elevated CO_2 and Drought on Proline Metabolism and Growth of Saf-flower (*Carthamus mareoticus* L.) Seedlings Without Improving Water Status

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Abstract: Plants subjected to ambient (350µmol mol^{-1}) and elevated (700µmol mol^{-1}) CO_2 were either well-watered or droughted. It was found that well-watered plants grown either in ambient or elevated CO_2 had the same water potential (Ψ_w), whereas the drought reduced (Ψ_w) especially in elevated CO_2. Leaves of elevated-CO_2 and well-watered grown plants contained more total soluble sugars, and starch contents than those of the ambient CO_2, whereas sucrose levels were similar for the well-watered plants of both elevated and ambient CO_2 treatments. In contrast, drought reduced the leaf starch and increased the total soluble sugars and sucrose contents in both CO_2 treatments. The activity of leaf sucrose phosphate synthase (SPS, EC: 2.4.1.14) of well-watered plants was not affected by high CO_2, whereas that of ambient-CO_2-grown plants was reduced 42 % by drought. Most of the amino acids showed an increase with the induction of drought and elevated CO_2, but proline only increased more markedly than did other amino acids, amounting to 37 folds compared to about 2 fold in ambient-CO_2-well watered grown plants. The activities of three enzymes, Δ^1-pyrroline-5-carboxylase reductase (P5CR, EC: 1.5.1.2) and ornithine aminotransferase (OAT, EC: 2.6.1.1.3) and proline dehydrogenase (PDH, EC: 1.5.1.2) were measured. The activity of P5CR and OAT was found to increase 6 and 3 folds respectively in high-CO_2 droughted plants. On the other hand, the activity of PDH was reduced by 40%. Accordingly, we concluded that proline accumulation in *Carthamus mareoticus* is caused by both the activation of its biosynthesis and inhibition of its degradation.

Key words: Saf-flower, (*Carthamus mareoticus* L.), pyrroline-5-carboxylic reductase, proline, dehydrogenase, relative water content, water potential

Introduction

It is well established that all plants have evolved many adaptations to grow, develop and reproduce in a narrow range of environmental conditions. Environmental stresses adversely affect these parameters in various crop plants. Understanding the physiological and biochemical mechanisms that make some species tolerant and others sensitive is fundamental in identifying clearly-the recognizable traits for use in breeding programs aimed at developing cultivars adapted to the environmental stress. Drought is considered as one of the most environmental factors that causes osmotic stress and limiting plant performance and growth as well as crop productivity and depends on the severity and duration of the drought period in many natural conditions.

Drought stress affects many physiological and biochemical processes in plants resulting in the alteration of some metabolic pathways. In terms of biochemical changes, several classes of compounds have been observed to accumulate in response to drought, among the major compounds are those involving carbohydrate metabolism, with the accumulation of neutral sugars, sugars alcohol (Delauney and Verma, 1993) and a number of other organic solutes in a wide range in various crop plants (Kameli and Losel, 1993). Also, drought-stress effects on plant nitrogen metabolism have been well documented. Major changes include enhanced proteolysis and inhibition of protein synthesis (Shen *et al*., 1990), associated with the accumulation and /or depletion of protein and non-protein amino acids, mainly proline and glycine betaine (Hanson and Hitz, 1982).

Accumulation of proline is a wide spread stress response, and it is one of the most frequently reported modifications induced by drought in plants because it appears to respond more profoundly than do most other amino acids and is often considered to be involved in stress resistance mechanisms (Lutts *et al*., 1999) through osmotic adjustment of stressed tissues (Kavi-Kishor *et al*., 1995), protection of enzymes (Solomon *et al*., 1994) and cellular structures (Van Rensburg *et al*., 1993) and acts as a free radical scavenger (Alia and Pardha, 1995). Moreover, proline biosynthesis occupies a central crossroad between carbon and nitrogen metabolism (Lutts *et al*., 1999). Proline degradation could occur through the action of proline dehydrogenase in the cytoplasm (PDH,EC:1.5.1.2).

Atmospheric CO_2 enrichment has a positive physiological impact on plants (Bowes, 1993). Several studies have shown that elevated CO_2 concentrations can partially alleviates the negative effects of drought on growth and improves plant water status by sustaining larger net CO_2 assimilation rates at reduced stomatal conductance in leaves of stressed plants (Rozema, 1993 and DeLuis *et al*., 1999), suggesting that increasing CO_2 concentration helps to avoid soil drought stress and yield loss caused by current ambient levels under water stress.

Saf-flower (*Carthamus mareoticus* L.) used in this study was a thistle like, annual plant of composite family, with large orange flower heads, and seeds that yield a drying oil used in paints, food and a dye-stuff or drug prepared from its florets. This plant is commonly found in the northwestern coastal desert of Egypt in Mareot area near Alexandria.

The objective of the current study was to evaluate the interactive effects of elevated CO_2 and drought on the plant growth, water relations, and some biochemical changes such as carbohydrate metabolism including the activity of sucrose phosphate synthase enzyme (SPS) and nitrogen metabolism. Furthermore, specific objectives were to determine the interactive effects of CO_2 enrichment and drought on the activity of some enzymes involved in proline metabolism such as P5CR, OAT and PDH to verify whether the accumulation of proline could be attributed to de novo synthesis and/or catabolism.

Materials and Methods

Growth conditions: Seeds of saf-flower (*Carthamus mareoticus* L.) were collected from the naturally-growing population in north western coastal desert of Egypt in Mareot area near Alexandria. Seeds were rinsed three times in distilled water and further surface sterilized by immersion for 20 min in H_2O_2 (3% v/v). Thereafter, the seeds were rinsed twice with distilled water, then germinated on two layers of filter paper moistened with deionized water in Petri dishes. Germination took place in darkness at 25°C. After 3 days, uniform seedlings were transferred to 2L plastic pots filled with washed sand (10 seeds per pot). Experiments were conducted in controlled growth chamber at 30/25°C day and night with a photoperiod of 16 hr. An average photosynthetic photon flux density was 400 µmol m^{-2} sec^{-1} at the leaf canopy level and the relative humidity was 75%. After emergence, seedlings were thinned to 3 plants per pot After 15 days, plants were randomly assigned to 4 treatments (5 pots per-treatment), each consisting one chamber: (1) well watered plants grown in ambient CO_2, (2) well watered plants grown in elevated CO_2, (3) plants grown with a soil water deficit (by withholding water) in ambient CO_2 and (4) elevated CO_2.

CO_2 exposure: CO_2 was supplied from a CO_2 cylinder gas purchased from commercial sources (Alexandria Air Product,. Alexandria, Egypt). Gas was delivered to the chambers through a manifold of in-line micrometer valves and flow meters air prior to entering the chamber to adjust for desired flow to the intake of two of four chambers. Due to the unavailability of continuous CO_2 monitoring, the concentration of CO_2 in each chamber was adjusted with pressure valves and monitored at the outlet of each chamber with barium hydroxide in the gas wash bottles titrated against 0.02N HCl using ph.ph as indicator and stirring the solution with CO_2-free air during the titration process. Mean daily CO_2 concentrations were 350 ± 5 and 700± 4 µmol mol^{-1} in ambient and elevated-CO_2 respectively.

Relative water content (RWC, %) and water potential (Ψ_w): Relative water content was determined using the method of Barrs and Weatherley (1962), and using the formula:

$$\frac{\text{(fresh weight - dry weight)}}{\text{(turgid weight – dry weight)}} \times 100$$

Turgid weight was determined after imbibition of the tissue in distilled water for 3 h. Dry weight was determined after incubation of the leaf segments in an oven at 80 °C for 48 hr.
Water potential was measured with a pressure chamber (Scholander *et al.*, 1965) in the youngest fully expanded leaves.
Water use efficiency (WUE) was determined as the ratio between the total dry matter production during stress period and the consumed water during the same period.

Determination of total soluble sugars, sucrose and starch: Total soluble sugars (TSS), sucrose and starch contents in leaves were quantified in 20 ml of 80% (V/V) ethanol extract at 95°C for 1 h from approximately 100mg of liquid nitrogen frozen leaf powder. After centrifugation at 10000g for 10min, starch was measured in the pellet according to Jarvis and Walker (1993). Soluble sugars were analyzed by reacting 0.25ml of the supernatant with 3 ml freshly prepared anthrone reagent (50 mg anthrone + 80.5 ml H_2SO_4 + 5ml H_2O) and placing in boiling water bath for 10 min. After cooling, the absorbance at 625 nm was determined according to Irigoyen *et al.* (1992). Sucrose was determined as the difference between reducing sugars before and after hydrolysis with 1 N HCl and 90°C for 30 min and neutralization. Starch content was determined according to the method of Murata *et al.* (1968).

Determination of protein and total free amino acids: The protein content and total free amino acids were quantified in 50 mM K-phosphate buffer (pH 7.5) extracts of fresh leaves (0.5 g). These extracts were filtered through four cheesecloth layers and centrifuged at 20000*g* for 30 min. Soluble protein was determined in the supernatant according to Bradford (1976) using bovine serum albumin as standard. The total free amino acids content was determined by the method of Sugano *et al.* (1975). The colour developed was estimated at 570 nm using L-leucine amino acid as standard.

Determination of proline: Fresh sample of leaf tissue (approximately 200 mg) was ground in liquid nitrogen and 1.2 ml of 3% (w/v) sulphosalicyclic acid to precipitate protein, under moderate shaking.
The homogenate was centrifuged at 12000g for 10 min. An aliquot (0.5 ml) of supernatant was made up to 1 ml with water. Proline was estimated in the supernatant by the method of Bates *et al.* (1973) using ninhydrin acid reagent and L-proline as standard (0-30 µg/ml)

Enzyme extractions: Leaves of well-watered and stressed plants were harvested and about 1 g fresh weight per treatment was then ground in a chilled mortar and pestle, in the presence of washed quartz sand with liquid nitrogen, then homogenized in the appropriate extraction buffer in a ratio 2:1, v/w at 4 °C.
Extraction buffer for SPS according to Vu *et al.* (1993) was 50 m*M* MOPS-NaOH buffer (pH 7.5) containing 15 mM $MgCl_2$, 1 m*M* EDTA, 2.5 mM DTT and 0.1%(v/v) Triton X-100. The homogenate was centrifuged at 12000*g* for 1 min at 2°C, and the supernatant was rapidly desalted by centrifugal filtration on Sephadex G-25 column.
Extraction buffer for ornithine aminotransferase (OAT) and Δ^1-pyrroline-5-carboxylic acid reductase (P5CR) enzymes contains 100 m*M* K-phosphate (pH 7.9)with pyridoxal phosphate (1mM), EDTA (1m*M*) and *β*-mercaptoethanol (10m*M*). The extract was filtered through cheesecloth and the filtrate was centrifuged for 10 min at 12000 *g*. The supernatant was used for the assay of OAT and P5CR enzymes. Extraction buffer for proline dehydrogenase (PDH) consisted of 100 mM K- phosphate (pH 7.4), 0.5% (v/v) Triton X-100. The extract was filtered through cheesecloth and the filtrate was centrifuged at 1000 *g* for 10 min. The supernatant was used for enzyme assay.

Enzyme assays: Activity of SPS was assayed in a reaction mixture containing 50 mM MOPS-NaOH (pH 7.5), 2.5 m*M* DTT, 15 m*M* $MgCl_2$, 3.5 mM UDP-glucose, 3.5 mM fructose-6-P and 15 m*M* glucose-6-P. After incubation for 10 min at 25 °C, the reaction was terminated with 70µl of 1 M NaOH and assay tubes were immersed in boiling water for 10 min to destroy all un-reacted fructose-6-P. After cooling, 0.5 ml of 0.1% (w/v) resorcinol in 95% ethanol and 1.5ml of 30% (v/v) HCl were added. The tubes were incubated at 80°C for 8 min, then cooled for 5 min in tap water, and absorbance was read at 520 nm.
The activities of ornithine aminotransferase (OAT), and Δ^1-pyrroline-5-carboxylic acid reductase (P5CR) enzymes were measured spectrophotometrically according to Charest and Phan (1990) and Lutts *et al.* (1999) respectively. The assay mixture for OAT enzyme contained Tris-KOH buffer (100mM, pH 8), ornithine (5 mM), α-ketoglutarate (10 mM), 0.25 mM NADH and enzyme (0.2 ml) in a total volume of 2ml; the decrease in absorbance of NADH was monitored at 340 nm. The assay mixture for P5CR enzyme contained 50 mM Tris-HCl buffer (pH 7.0),1mM dithiothreitol, 0.25 mM NADH and 1mM Δ^1-pyrroline-5-carboxylic acid (P5C). The reaction was started with the addition of 0.1 ml crude extract and the decrease in the absorbance of NADH was monitored at 340 nm.
Proline dehydrogenase (PDH) was assayed according to Mattioni *et al.* (1997) by following the reduction of NAD^+ at 340 nm in Na_2CO_3-$NaHCO_3$ buffer (200 mM, pH 10.3) containing L-proline (15 mM); NAD^+ (10 m*M*) and enzyme extract (0.1 ml) in a total volume of 0.5ml.

Statistical analysis: Analysis of variance (ANOVA) was performed to partition the variance into the main effects and the interaction between the two factors (CO_2 and drought). Data were subjected to Dunnet's t-test to find statistically the significant differences between treatments at $P \leq 0.05$. The data shown are means ± SE.

Results and Discussion

A sufficient supply of water is fundamental for vegetation diversity, optimal growth, and plant productivity. Therefore, drought stress may affect some physiological processes, resulting in marked decrease in the yield of plants. Also, high CO_2 grown plants usually deplete soil water at a lower rate than ambient CO_2 grown plants and, therefore, slow the rate of stress development without subjecting the plants to the same of soil drought as ambient grown plants (De Luis *et al.*, 1999). Well-watered plants grown either in ambient or in elevated CO_2 had the same water potential (Ψ_w) and relative water content (RWC) (Fig. 1). In contrast, plants grown under drought conditions displayed a more rapid and a significant reduction in Ψ_w and RWC of saf-flower plant grown in ambient CO_2 more than those grown in elevated CO_2, indicating that CO_2 can partially alleviate the detrimental

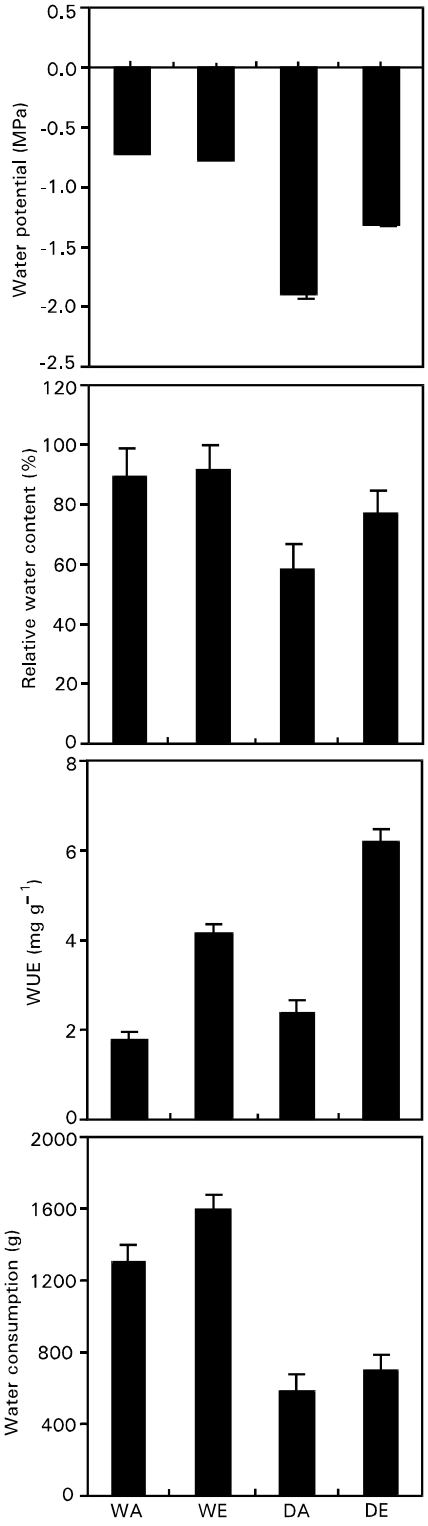

Fig. 1: Effect of the interaction between elevated CO_2 and drought on water potential, relative water content, water consumption and water use efficiency (WUE). WA and EW, well-watered *Carthamus mareoticus* L. plants grown in ambient (350 µmol mol^{-1}) or in elevated CO_2 (700 µmol mol^{-1}). DA and DE, plants grown in drought under ambient and elevated CO_2 conditions. Each column represents the mean ± SE (n= 10)

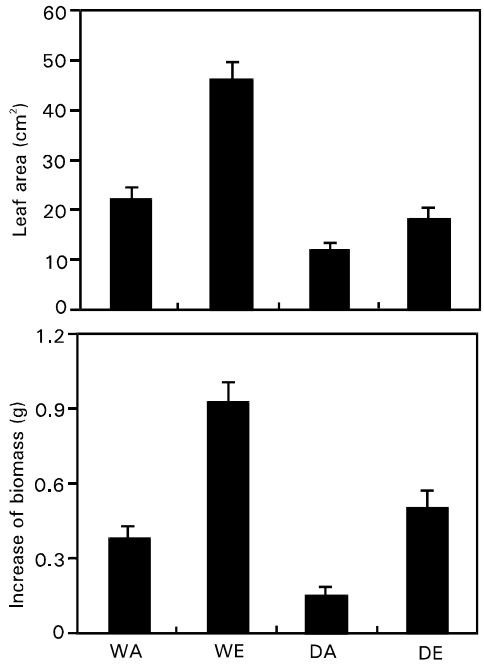

Fig. 2: Effect of the interaction between drought and elevated CO_2 on the leaf area and biomass of *Carthamus mareoticus* L. plant values are mean ± SE (n= 10). Otherwise as for Fig. 1

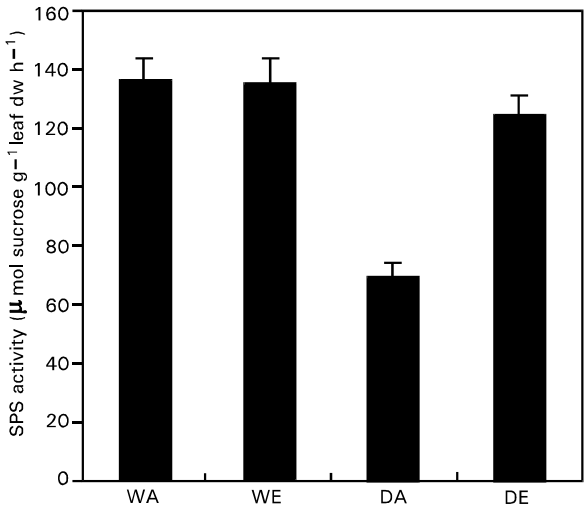

Fig. 3: Effect of the interaction between drought and elevated CO_2 on the activity of SPS enzyme in leaves of *Carthamus mareoticus* L. plant. Values are mean± SE. Otherwise, as for Fig. 1.

effect of drought through its effect on plant water status, rather than reduction in hydraulic conductance as suggested by De-Luis *et al.* (1999). CO_2 enrichment also enhances water conservation and midday xylem water potentials in drought-stressed plants such as sweet potato (Bhattacharya *et al.*, 1990) and *Arachis hypogaea* (Clifford *et al.*, 1993). Furthermore, Jarvis (1989) suggested that elevated CO_2 could improve drought tolerance by increasing rates of photosynthetic assimilation, thus resulting in accumulation of organic solutes as osmolytes such as soluble sugars, as we also obtained in this study (Table 1), which causes

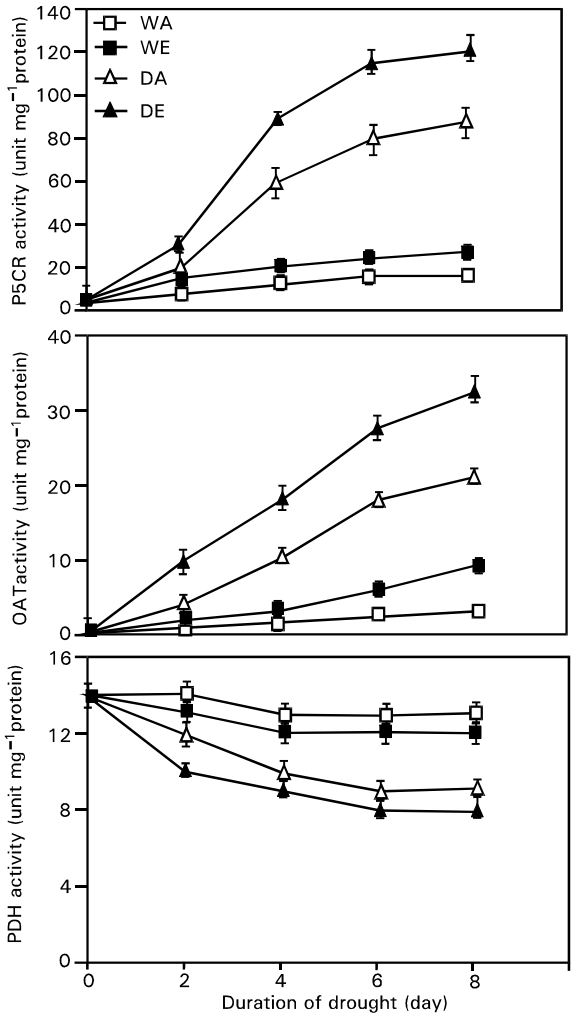

Fig. 4: Effect of the interaction between drought and elevated CO_2 on the activity of pyrroline-5-carboxylic reductase (P5CR), omithine amino transferase (OAT) and Proline dehydrogenase (PDH) enzymes in Carthamus mareoticus L. plant. Unit activity = the amount of enzyme catalyzing the oxidation/reduction of mM of NADH/NAD min−1 and referred to the protein content of each sample. Otherwise as for Fig. 1

water to be retained and keeps the Ψ_w gradient less negative (Delauney and Verma ,1993) and continue uptake of water during stress period and it is expected that this will counteract the negative effect of water stress on biomass accumulation (Carter *et al.*, 1997).

In the present study, it was found that a higher water use efficiency (WUE) in stressed plants was more than in well-watered plants, probably due to the lower water consumption (drought effect), also elevated CO_2 increased this difference (Fig. 1). Most studies in drought-subjected plants growing in elevated CO_2 have also found an increase in WUE (Bazzaz, 1990 and Chaudhuri *et al.*, 1990) associated with a decrease in water consumption. However in well-watered plants the increases in WUE with elevated CO_2 was associated with high water consumption (Fig. 1). According to Samarakoon and Gifford (1996), the reason for WUE improvement in well–watered plants under elevated CO_2 was the higher biomass production rather than a decrease in water consumption. On the other hand, Chaves and Pereira (1992) concluded that the increase in WUE as a result of elevated CO_2 is likely to be the improvement in well watered plants more than the increase in net photosynthesis only, and that the same for drought grown plants grown in a CO_2 –enriched atmosphere. Furthermore, stomata are known to play an important role in mediating the response of plants to drought and CO_2 concentration. For most plant species, drought frequently causes stomata closure, also increased atmospheric CO_2 that causes partial closure and reduces conductance of the stomata (Drake *et al.*, 1997) with the reduction of water loss through transpiration. Such reduction in stomatal aperture, conductance and transpiration explain the better water status of the plants grown in elevated CO_2.

Table 1: Effect of interaction between CO_2 and water availability on starch, sucrose and total soluble sugar content in *Carthamus mareoticus* leaves at the end of treatments. Data represents the means ± SE (n= 5). WA and WE, well-watered plants grown in ambient and elevated CO_2. DA and DE, plants grown under drought conditions in ambient and elevated CO_2

Treatments	Starch (mg g^{-1} DW)	Sucrose (mg g^{-1} DW)	Total soluble sugars (mg g^{-1} DW)
WA	110.6± 9.9	40.8± 4.4	112.8± 6.7
WE	210.9± 9.8	41.6± 3.4	159.2± 6.8
DA	85.8± 4.6	53.9± 2.9	79.9± 5.4
DE	126.4± 6.5	62.8± 5.8	109.8± 8.8

Table 2: Effect of interaction between CO_2 and water availability on protein, free amino acid and proline content in *Carthamus mareoticus* leaves at the end of treatments. Data represents the means ± SE (n= 5). Otherwise as for Table 1.

Treatments	Total soluble protein (mg g^{-1} DW)	Total free amino acids (µg g^{-1} DW)	Proline (µg g^{-1} DW)
WA	221.8± 9.8	1010± 22.7	43± 2.6
WE	219.7± 8.2	1161± 33.8	52± 4.2
DA	151.5± 6.6	1860± 28.5	679± 11.8
DE	136.9± 8.1	3104± 46.6	1594± 35.7

The drought significantly depressed the dry weight (DW) compared with well-watered plants (control) (Fig. 2). However, in elevated CO_2, the increase in biomass production of well-watered or even in stressed plants was higher than in ambient CO_2, probably due to the better assimilating efficiency of their tissues (increase in net assimilation) and to the better performance of plants tissues, thus resulting in high dry matter production particularly in well-watered plants. In this connection, Huber *et al.* (1984) have reported that the beneficial effect of elevated CO_2 on growth of stressed plants may be related to a better plant water status. Idso (1988) indicates in his study on a variety of plant species that atmospheric CO_2 enrichment may actually prevent plants from succumbing to the rigors of environmental stresses and enable them to maintain essential growth. CO_2 significantly increased the total leaf area particularly in well-watered plants (Fig 2). Similar situations have been reported for plant species (Weber *et al.*, 1994 and Edward *et al.*, 1997).

Metabolic changes: Drought and elevated CO_2 are known to trigger several metabolic changes in plants particularly in leaves. Determinations of starch content for the treatments showed that elevated CO_2 affected the concentration of starch in saf-flower (*Carthamus mareoticus*) leaves (Table 1). Leaf starch content of well watered plants under elevated CO_2 conditions (WE) was 91% higher than that of plant under ambient CO_2 (WA). On the other hand, drought reduced leaf starch content of DA plants to about 22 % of that of the WA plants, but under elevated CO_2 (DE plants) it declined strongly to about 40% of that of the WE plants.

As expected in this study, under well-watered conditions, elevated CO_2 significantly increased total soluble sugars content of leaves, but drought reduced it in elevated and ambient CO_2 and no differences were observed between DE and WA treatment (Table 1), possibly due to increase in photosynthetic assimilation and a decrease in respiration rates (Long, 1991), which increased the

availability of photosynthates (DeLuis *et al.*, 1999). Many authors have found that carbohydrate accumulates significantly under elevated CO_2 (Sage *et al.*, 1989 and Yelle *et al.*, 1989 a,b). Sucrose content was similar in leaves of the well-watered plants under conditions of both ambient and elevated CO_2. However, there were increases in sucrose content in the leaves of droughted plants. Leaf sucrose of the DA plants was 32 % and increased by about 51 % in DE relative to the content of both WA and WE plants respectively. The increase in sucrose content with a concomitant decrease in starch content could be due to the conversion of starch into sucrose (Chaves, 1991).

Changes observed in soluble protein, free amino acid, proline content of several drought stressed plant species have been attributed to a reduction in the rates of protein synthesis and an increase in proteolytic activity both of which tend to cause an increase in the total soluble nitrogen (Shen *et al.*, 1990). In the present study, drought resulted in a decrease in total soluble protein. However, we did not observe changed soluble protein content due to elevated CO_2 in well watered plants. The present data also show a significant increase in free amino acids (Table 2). These results would suggest that the decrease in the soluble protein content cannot be related to the increase in amino acids but could be due to the reduction in protein synthesis rather than the initiation of proteolysis as previously shown in *Brassica napus* and wheat seedlings by Good and Zaplachinski (1994) and Mattioni *et al.* (1997) respectively.

Although the accumulation of free amino acids showed a significant increase under drought conditions and elevated CO_2 amounted to nearly 3 folds, only the proline content increased to 37 folds under the identical conditions (Table 2). Thus, proline was high enough to be considered the principal solute that may allow plants to overcome drought effect through osmotic adjustment and serves as storage forms of nitrogen and carbon for future use under less stressful conditions. A function of proline as non-protein amino acid in osmo-adjustment has been proposed, although there may be no cause and effect relationship between proline accumulation and osmo-regulation in plants grown under drought conditions and responses of plants suggested by differences in proline concentrations and responses of plants species to drought (Sundaresan and Sudhakaran, 1995). However, the accumulation of proline during drought may have other functions, such as enzyme protection and stabilization of biological membranes and the degradation of proline may improve the energy status of cells recovering from water deficit (Alia, 1991; Mattioni *et al.*, 1997)

Activity of SPS enzyme: Drought stress was found to reduce the leaf sucrose phosphate synthase (SPS) activity, whereas the reduction of SPS activity was prevented by elevated CO_2 (Fig. 3). However, elevated CO_2 did not affect the activity of SPS under well-watered conditions. Furthermore, SPS activity of DA plants was 51% of that of the WA plants, indicating that SPS was not down regulated by elevated CO_2. This might be expected in that SPS would be required to handle the increased carbohydrate amounts at elevated CO_2. Similar results have been reported by Vu *et al.* (1998) in rice. They reported that although the elevated CO_2 treatment prevented the drought induced reduction in SPS activity, it did not prevent a major conversion of starch to soluble sugar particularly sucrose in drought stressed plants, but only delayed the onset of this process. Thus, the high sucrose content increased as a result of drought in both growth CO_2 regimes, despite the fact that SPS activity declined under ambient CO_2.

Activities of enzymes related to proline metabolism: In order to evaluate whether the proline accumulation in plants subjected to drought stress is an active process brought about by the stress or not, the activities of the Δ^1-pyrroline-5-carboxylase reductase (P5CR), ornithine aminotransferase (OAT), and proline dehydrogenase (PDH) enzymes involved in biosynthesis and catabolism of proline, were measured in well-watered and droughted plants under ambient and elevated CO_2 conditions. OAT, as the key enzyme in pathway involves the conversion of ornithine, which is also the precursor of putrescine, to the formation of glutamate semi-aldehyde which spontaneously converts by P5CR to pyrroline-5-carboxylate, the precursor of proline. PDH, on the other hand, is the key enzyme involved in degradation of proline. However, there are conflicting data in the literature concerning the quantitative implication of these enzymes in proline biosynthesis. Most of the studies on drought stress induced proline accumulation have examined only the glutamate pathway of proline metabolism.

The present study showed that under well-watered conditions, elevated CO_2 slightly increased P5CR activity, whereas drought caused a significant increase in the activity of this enzyme. There was a significant difference in activity between well-watered and droughted plants grown under ambient and elevated CO_2, where the increase in P5CR activity observed in droughted plants was around 18 and 24 folds in ambient and elevated CO_2 respectively compared to 3 and 4 folds in WA and WE respectively (Fig. 4), which associated with proline accumulation (Table 2) indicating that glutamate pathway is considered the predominant and an important mechanism in biosynthesis of proline in saf-flower (*Carthamus mareoticus*) plants grown under drought conditions in ambient or elevated CO_2. Similar results have been reported in other crop species (Roy *et al.*, 1992; Strizhov *et al.*, 1997 and Lutts *et al.*, 1999). Also, drought induced stimulation of OAT constitutes an interesting result since it was detected and increased only in proline accumulating plants in response to drought and elevated CO_2 (Fig. 4). Hervieu *et al.* (1994) and Roosens *et al.* (1998) have showed that OAT activity increased in radish cotyledons and in young plants of *Arabidopsis thaliana* respectively after exposure to water stress. It could therefore be suggested that the stress-induced stimulation of OAT activity would simultaneously lead to an increase in proline concentration in drought grown plants and to a decrease in endogenous levels of the protective polyamine, putrescine (Lutts *et al.*, 1999). Elevated CO_2 increased proline accumulation due to the increase in the endogenous pool of its precursor glutamate. Furthermore, the reduction in the activity of PDH, as a key enzyme in proline catabolism, to reach the minimum level in stressed plants (Fig. 4) may also be implicated (Sundaresan and Sudhakaran, 1995) suggesting that PDH enzyme is to be one of the factors causing proline accumulation.

However, a lack of relationship between proline accumulation and P5CR or PDH activities was reported in several plant systems (LaRosa *et al.*, 1991 and Szoke *et al.*, 1992). Moreover, Mattioni *et al.* (1997) showed that although the level of P5CR activity was increased in the leaves of wheat stressed plants, no change was observed in the expression of the *AT-P5CR1* , implying that the increase in enzyme activity is not related to changes in the expression of this gene.

Our study shows that the well-watered and elevated CO_2 conditions improved growth and this effect was due to a better performance of plant tissues. Under drought condition, elevated CO_2 alleviated the negative effects of drought and improved tolerance by improving carbon fixation and plant growth. As a result, plant biomass production was even higher than in well-watered plants grown in ambient CO_2. In addition, we have shown that in saf-flower (*Carthamus mareoticus*) plant proline accumulation under drought stress is an active process that requires the activation of biosynthetic, mainly P5CR , and catabolic enzymes such as PDH.

References

Alia, K.V.S.K.P. and S.P. Pardha, 1995. Effect of zinc on free radicals and proline in Brassica and Cajanus. Phytochemistry, 39: 45-47.

Barrs, H.D. and P.E. Weatherley, 1962. A re-examination of the relative turgid technique for estimating water deficit in leaves. Aust. J. Biol. Sci., 15: 413-428.

Bates, L.S., R.P. Waldren and I.D. Teare, 1973. Rapid determination of free proline for water-stress studies. Plant and Soil, 39: 205-207.

Bazzaz, F.A., 1990. The response of natural ecosystems to the rising global CO_2. Annu. Rev. Ecol. Syst., 21: 167-196.

Bhattacharya, N.C., D.R. Hileman, P.P. Ghosh, R.L. Musser, S. Bhattacharya and P.K. Biswas, 1990. Interaction of enriched CO_2 and water stress on the physiology of and biomass production in sweet potato grown in open top chambers. Plant Cell Environ., 13: 933 - 940.

Bowes, G., 1993. Facing the inevitable: Plants and increasing atmospheric CO_2. Ann. Rev. Pl. Physiol. Pl. Mol. Biol., 44: 309-332.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.

Carter, E.B., M.K. Theodorous and P. Morris, 1997. Responses of hange. I. Effects of elevated CO_2 temperature and drought on growth and plant development. New Phytol., 136: 245-253.

Charest, C. and C.T. Phan, 1990. Cold acclimation of wheat (*Triticum aestivum*): Properties of enzymes involved in proline metabolism. Physiol. Pl., 80: 159-168.

Chaudhuri, U.N., M.B. Kirkham and E.T. Kanemasu, 1990. Carbon dioxide and water level effects on yield and water use of winter wheat. Agron. J., 82: 637-641

Chaves, M., 1991 Effects of water deficits on carbon assimilation. J. Exp. Bot., 42: 1-16

Chaves, M . and J. Pereira, 1992. Water stress, CO_2 , and climate change. J. Exp. Bot., 43: 1131-1139.

Clifford, S.C., I.M. Stronach, A.D. Mohamed, S.N. Azam-Ali and N.M.J. Crout, 1993. The effects of elevated atmospheric carbon dioxide and water stress on light interception, dry matter production and yield in stands of groundnut (*Arachis hypogaea* L.). J. Exp. Bot., 44: 1763-1770.

Delauney, A. and D. Verma, 1993. Proline biosynthesis and osmoregulation in plants. Pl. J., 4: 215-223.

De-Luis, I., J.J. Irigoyen and M. Sanchez-Diaz, 1999. Elevated CO_2 enhances plant growth in droughted N_2- fixing alfalfa without improving water status. Physiol. Pl., 107: 84-89.

Drake, B.G., M.A. Gonzalez-Meler and S.P. Long, 1997. More efficient plants: A consequence of rising atmospheric CO_2 . Annu. Rev. Pl. Physiol. Pl. Mol. Biol., 48: 609- 639.

Edward, H., R.C. Pausch, R.A. Rowland, C.L. Mulchi and B.F.T. Dorff, 1997. Responses of field-grown soybean (cv. Essex) to elevated SO_2 under two atmospheric CO_2 concentrations. Enivron. Exp. Bot., 37: 85-93.

Good, G.A. and S.T. Zaplachinski, 1994. The effects of drought stress on free amino acid accumulation and protein synthesis in *Brassica napus*. Physiol. Pl., 90: 9-14.

Hanson, A.D. and W.D. Hitz, 1982. Metabolic responses of mesophytes to plant water deficits. Ann. Rev. Pl. Physiol., 33: 163-203.

Hervieu, F., F. LeDily, J-P. Billard and C. Huault, 1994. Effects of water stress on proline content and ornithine aminotransferase activity of radish cotyledons. Phytochemistry, 37: 1227-1231.

Huber, S.C., H.H. Rogers and F.L. Mowry, 1984. Effects of water stress on photosynthesis and carbon partitioning in soybean (*Glycine max* L. Merr.) plants grown in the field at different CO_2 levels. Pl. Physiol., 76: 224-249

Idso, S.B., 1988. Three phases of plant response to atmospheric CO_2 enrichment. Pl. Physiol., 87: 5-7

Irigoyen, J.J., D.W. Emerich and M. Sanchez-Dias, 1992. Water stress induced changes in concentration of proline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. Physiol. Pl., 84: 55-60.

Jarvis, P.G., 1989. Atmospheric carbon dioxide and forests. Philos. Trans R. Soc., 324: 369-392.

Jarvis, C.E. and J.R.L. Walker, 1993. Simultaneous, rapid, spectrophotometric determination of tota starch, amylose and amylopectin. J. Sci. Food Agric., 63: 53-57.

Kameli, A. and D.M. Losel, 1993. Carbohydrates and water status in wheat plants under water stress. New Phytol., 125: 609-614.

Kavi-Kishor, P.B., Z. Hong, G.H. Miao, C.A.A.Hu, and D.P.S. Verma, 1995. Over expression of Δ^1- pyrroline-5-carboxylate synthetase increase proline production and confers-otolerance in transgenic plants. Pl. Physiol., 108: 1387-1394.

LaRosa, C., D. Rhodes, J.C. Rhodes, R.A. Bressan and L.N. Csonka, 1991. Elevated accumulation of proline in NaCl-adapted tobacco cells is not due to altered, Δ^1-pyrroline-5-carboxylase reductase. Pl. Physiol., 96: 245-250.

Long, S.P., 1991. Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO_2 concentrations. Has its importance been underestimated. Plant Cell Environ., 14: 729-739.

Lutts, S., V. Majerus and J.M. Kinet, 1999. NaCl effects on proline metabolism in rice (*Oryza sativa*) seedlings. Physiol. Pl., 105: 450-458.

Mattioni, C., N.G. Lacerenza, A. Troccoli, A.M. De-Leonardis and N.Di Fonzo, 1997. Water and salt stress-induced alterations in proline metabolism of *Triticum durum* seedlings. Physiol. Pl., 101: 787-792.

Murata, T., T. Akazawa and F. Shikiko, 1968. Enzymic mechanism of starch breakdown in germinating rice seeds. Pl. Physiol., 43: 1899-1911.

Roosens, N.H.C.J., T.T. Thu, H.M. Iskandar and M. Jacobs, 1998. Isolation of the ornithin-ä-aminotransferase cDNA and effect of salt stress on its expression in *Arabidopsis thaliana*. Pl. Physiol., 117: 263-271.

Roy, D., N. Bhunia, N. Basu and S.K. Banerjee, 1992. Effect of NaCl salinity on metabolism of proline in salt-sensitive and salt resistance cultivars of rice. Biol. Pl., 34: 159-162.

Rozema, J., 1993. Plant responses to atmospheric carbon dioxide enrichment: Interactions with some soil and atmospheric conditions. Vegetatio, 104/105: 173-193

Sage, R.F., T.D. Shakey and J.R. Seeman, 1989. Acclimation of photosynthesis to elevated CO_2 in five C_3 species. Pl. Physiol., 89: 590-596.

Samarakoon, A.B. and R.M. Gifford, 1996. Water use and growth in response to elevated CO_2 in wet and drying soil. Aust. J. Pl. Physiol., 23: 491-500.

Scholander, P.F., H.T. Hammel, D. Bradstreet and E.A. Hemmingse, 1965. Sap pressure in vascular plants. Science, 184: 339-346.

Shen, L., D.M. Orcutt and J.G. Foster, 1990. Influence of drought on the concentration and distribution of 2,4-diaminobutyric acid and other free amino acids in tisues of flatpea (*Lathyrus sylvestris* L.). Environ. Exp. Bot., 30: 497-504

Solomon, A., S. Beer, Y. Waisel, G.P. Jones and L.G. Paleg, 1994. Effects of NaCl on the carboxylating activity of Rubisco from *Tamarix jordanis* in the presence and absence of proline related compatible solutes. Physiol. Pl., 90: 198-204

Strizhov, N., E. Abraham, L. Okresz, S. Blicking, A. Ziberstein, J. Schell, C. Koncz and L. Szabados, 1997. Differential expression of two *P5CR* genes controlling proline accumulation during salt-stress requires ABA and is regulated by *ABA1, AB II and AXR2* in Arabidopsis. Pl. J., 12: 557-559

Sugano, N., T. Tanaka, E. Yamamoto and Andnishi, 1975. phenylalanine ammonialyase in carrot cells in suspension cultures. Phytochemistry, 14: 2435-2440.

Sundaresan, S. and P.R. Sudhakaran, 1995. Water stress-induced alterations in the proline metabolism of drought-susceptible and –tolerant cassava (*Manihot esculenta*) cultivars. Physiol. Pl., 94: 635-642.

Szoke, A., G.H. Miao, Z. Hong and D.P.S. Verma, 1992. Subcellular location of Δ^1-pyrroline-5-carboxylase reductase in root/nodule and leaf of soybean. Pl. Physiol., 99: 1642-1649.

Van Rensburg, L., G.H.J.J. Kruger and H. Kruger, 1993. Proline accumulation as drought- tolerance selection criterion: its relationship to membrane integrity and chloroplast ultrastrcture in *Nicotiana tabacum* L. J. Pl. Physiol., 141: 188-194.

Vu, J.C.V., R.P. Niedz and G. Yelenosky, 1993. Glycrol stimulation of chlorophyll synthesis, embryogenesis, and carboxylation and sucrose metabolism enzymes in nucellar callus of Hamlin' sweet orange. Plant Cell Tissue Organ Cult., 33: 75-80.

Vu, J.C.V., J.T. Baker, A.H. Pennanen, L.H. Allen, G. Bowes and K.J. Boote, 1998. Elevated CO_2 and water deficit effects on photosynthesis, ribulose biphosphate carboxylase-oxygenase, and carbohydrate metabolism in rice. Physiol. Plant, 103: 327-339.

Weber, J.A., D.T. Tingey and C.P. Anderson, 1994. Plant response to air pollution. In: Wilkinson, R.E. ed., Plant environment Interactions. Marcel Dekker.New York, Basel, Hong Kong, pp: 339-357.

Yelle, S., R.C. Beeson, M.J. Trudel and A. Osselin, 1989a. Acclimation of two tomato species to high atmospheric CO_2 .I. Sugar and starch concentration. Pl. Physiol., 90: 1465-1472.

Yelle, S., R.C. Beeson, M.J. Trudel and A. Gosselin, 1989b Acclimation of two tomato species to high atmospheric CO_2. II. Ribulose-1.5-biphosphate carboxylase/oxygenase and phosphoenolpyruvate carboxylase. Pl. Physiol., 90: 1473-1477.