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Research Article

Effect of Elevated Carbon Dioxide on Growth, Photosynthesis Pigments and Abscissic Acid Content in Phosphoribulokinase Deficient Transgenic Tobacco Plants Grown Under Drought Condition

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Study investigate effect of ambient (350 μmol mol^{-1}) and elevated (700 μmol mol^{-1}) CO_2 under water stress conditions on phosphoribulokinase deficient transgenic tobacco plants (C8) generated through antisense technique and found to have only 5% phosphoribulokinase activity (referred to as transformants) as compared to wild type (Bin-19) on growth, net CO_2 assimilation, chlorophyll fluorescence, photosynthetic pigments, ABA content and activity of peroxidase. CO_2 enrichment resulted in significant increase in biomass in transformant and wild type plants and almost completely mitigated the effect of water stress on biomass. Rate of net photosynthesis (Pn) was also higher in plants grown at elevated CO_2 irrespective of wild or transformant and showed even greater Pn even under water stress conditions. Stomatal conductance was also higher in plants grown at 700 μmol mol^{-1} CO_2 level and decreased relatively to lesser extent even when water stressed than seen at ambient CO_2 level. There was no change in chlorophyll fluorescence parameters (Fv/Fm, qP and qN) in wild or transformant plants nor water stress influenced the fluorescence. However, CO_2 enrichment resulted in slight increase in qP and qN. Peroxidase activity was greater in wild type plants. Elevated level of CO_2 further increased the level of Peroxidase activity in both wild as well as in transgenic plants. Plants experiencing water stress showed even higher level of peroxidase activity. Wild type plants showed higher level of pigments including xanthophylls compared to transformants. CO_2 enrichment did not affect the pigment content appreciably. De-epoxidation of V to Z was greater in wild type plants than seen in transformants. Ambient level of CO_2 resulted in better de-epoxidation of V to Z than CO_2 enrichment in both type of plants. ABA content showed very little difference in wild type or transformant plants grown either at elevated or ambient level of CO_2. However, plants subjected to water stress increased the level of ABA many fold in both transformants as well as in wild type plants irrespective of level of CO_2 they were grown. The data presented here show that plants grown at elevated level of CO_2 resulted in better growth and better water stress tolerance. C8 plants showed relatively more sensitivity to stress which could be result of substrate (RUBP) limitation, as a result of 95% less of PRK enzyme resulting in low carbon metabolism as well as over energization (reduction) of the photosynthetic electron transport chain.

From preindustrial levels of 280 μmol mol^{-1} the global atmospheric CO_2 concentration has risen by roughly 28% to the current level of 360 μmol mol^{-1} (McKee *et al.*, 1997), and is expected to exceed 600 μmol mol^{-1} by the middle of 21st century (IPCC, 1994). It is proposed that such an increase in atmospheric CO_2 and other green house gases will have a significant impact on global climate. Most global climate model predict that doubling of CO_2 level will increase atmospheric temperature by 3-5°C resulting in drought condition in addition to the effect on global temperature. The increase in the atmospheric CO_2 level may stimulate photosynthesis directly, specially in C3 plants, through its effect on plant photosynthesis and stomatal behavior, but high temperature resulting in drier environment may cause decrease in the photosynthesis. The prevailing

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prove beneficial to many farmers and foresters. The assumption is that plants need atmospheric CO_2 to produce food (i.e. carbohydrate) and excess CO_2 will act as nutrition to plants which will cause some crops and trees to grow bigger and faster. But Schwartz (2002) had raised question about this assumption and reported that elevated atmospheric CO_2 may reduce plant growth when combined with other likely consequences of climate change namely high temperature and drought or increased precipitation.

Effect of elevated CO_2 in combination with drought condition is of particular importance since many region could experience an increased frequency of drought caused by climate change and may have effect on food security of the country. There are report of instantaneous enhanced water use efficiency in elevated CO_2 in C3 plants, but this may not necessarily lead to increased drought tolerance (Heath and Kersteins, 1997; Morrison, 1993). It is conceivable that elevated CO_2 might influence factors such as stomatal conductance or leaf area in such a way as to reduce tolerance to drought in some species. The potential for enhanced growth and productivity in elevated CO_2 and the associated feed back to the global carbon cycle could be affected on water balance. Even a slight reduction in drought tolerance could contribute to long term decline. It is therefore, necessary to understand how plants will respond to such a CO_2 enrichment and water stress conditions. In light of possible precipitation shift caused by global warming and concern about changing environment and productivity determining the direct and possible interactive effect of CO_2 with drought stress becomes extremely important. In this study we attempt to learn the effect of elevated CO_2 under water stress conditions in transgenic tobacco plants genetically modified to have approximately 5% of Phosphoribulosekinase enzyme which take part in metabolising Ribulose 5 phospahte to Ribulose 1,5-bisphosphate, a substrate for CO_2 fixation, and also likely to cause over reduction of the electron transport chain.

MATERIAL AND METHODS

Tobacco (*Nicotiana tabacum* L) plants were used in this study. Tobacco was transformed with *Agrobacterium tumefaciens* containing either the binary vector pBIN19 producing plants with wild type concentrations of phosphoribulo kinase (PRK), referred to as wild-type or pBIN19 with antisense PRK construct producing plants with only 5% of wild type PRK activity, referred to as transformant or C8; Paul *et al.* (1995). Plants were grown as described by Habash *et al.* (1996). Seeds were sown and germinated at 25°C on filter paper in petridishes and transferred to a peat-based soil (Effcompost; Croxdens, UK) and grown in two different 6 feet x 8 feet x 6 feet controlled environmental chambers, one having CO_2 level of 350 µmol mol^{-1} (ambient) and another chamber having CO_2 level of 700 µmol mol^{-1} (elevated) and all other environmental conditions being identical of 25°C day/night temperature, 80% RH and 280-340 µmol m^{-2} s^{-1} quantum flux measured at the uppermost leaf. Third leaf from top of 6 week old plants were used for all the measurements.

Water stress and its measurement

Plants of same age were exposed to different level of water stress by withholding water to plants for different duration. Water potential was measured using Bomb pressure meter.

Measurement of Relative water content

Relative water content (RWC) was measured using formula of (Fresh weight-dry weight)/(turgor weight-dry weight) x 100. Turgor weight was measured by floating the leaf disc on distilled water for over 6 hours under diffuse light. The disc was dried gently with tissue paper and weight taken on digital balance.

Gas exchange measurements

Gas exchange was measured on attached leaves using a multichamber open-circuit system according to Habash *et al.* (1995). Calculation of assimilation, stomatal conductance were based on Von Caemmerer and Farquhar (1981).

Fluorescence Measurements

Room temperature PS II chlorophyll fluorescence was measured at steady state photosynthesis on intact attached leaves in a specially designed gas exchange chamber allowing measurements of gas exchange and fluorescence simultaneously as described by Habash *et al.* (1995). The following fluorescence parameters were measured: Maximal fluorescence induced by saturating pulse at steady state photosynthesis (Fm'), maximal fluorescence induced by a saturating pulse after 15 min dark adaptation (Fm), steady state fluorescence under continuous illumination (Fs), minimal level of fluorescence at steady state fluorescence (F'o) and minimal level of fluorescence after 15 min of dark adaptation (Fo). The relative quantum yield of PS II electron transport (ϕPSII) is defined as (Fm'-Fs)/FM according to Genty *et al.* (1989), photochemical quenching of fluorescence (qP) as (Fm'-Fs)/(Fm'-Fo') and non photochemical quenching of fluorescence (qN) as 1-(Fm'-Fo')/(Fm-Fo) according to Schreiber *et al.* (1986).

3A estimation

af tissue (1 g) was thoroughly extracted in 1 ml of 0% acetone containing 0.1% butyl hydroxy ludene (BHT) in 1.5 ml eppendorf tube. The extract as incubated on ice for 2 h and then centrifuged at 00 g for 20 min at 4°C. The supernatant was filtered rough 45 µm filter. Only 15 µl of the filtrate was sed for ABA estimation using (+)ABA specific nnoclonal antibody MAC252 procured from S.A. arrie, John Innes Centre, Norwich, U.K.; according the method described by Quarrie *et al.* (1988). To e sample 100 µl of antibody, 15 µl of MAC252 in 5 ml of BSA/PVP solution and 100 µl of H3 ABA d 200 µl of PBS buffer was added and the mixture as incubated for 45 min at 4°C. To this solution 0 µl of saturated $(NH_4)_2 SO_4$ was added and further ncubated for 30 min at room temperature and entrifuged. Pallet was dried and dissolved in 1 ml f 50% $(NH_4)_2 SO_4$ solution and again centrifuged. Pallet was again dried and redissolved in 100 µl of istilled water and to this 1.2 ml of scintillant was dded. The vial were placed inside the 20 ml cintillation vials and counts were taken for 10 min. The concentration was calculated by counting the ount with standards of ABA.

Pigment analysis

HPLC analysis of pigments was carried out according to Sharma and Hall (1992). Fresh leaves (1 g) were frozen in liquid nitrogen and homogenised in a pestle and mortar in 5 ml methanol in the dark. The homogenate was centrifuged for 5 min at 10,000 rpm at 4°C. The methanol was evaporated from the sample using N_2 gas and sample were stored at –70°C for HPLC analysis. Identification and separation of carotenoids was carried out using HPLC with a reverse phase column (Phenomenix-Hypersil 5 C18 (250 x 3.2 mm) and Milton-Roy spectromonitor 3000 variable wavelength detector using Trivector Trio chromatogrpahy computing integrator. The sample were redissolved in 200 µl of methanol and 100 µl of this was injected into the HPLC. The gradient for separation was 0-100% methyl acetate in acetonitrile:water (9:1) over 30 min at 1.0 ml/min flow rate. The peaks were identified using standards and spectra of individual peaks.

Peroxidase measurements

Peroxidase activity was monitored according to Sankhalkar and Sharma (2002). Activity was assayed in a reaction mixture containing guaicol (3.4 mM), H_2O_2 (0.9 mM) and potassium phosphate (50 mM; pH 6.0) and leaf extract equivalent of 50 mg of protein. The mixture was incubated for 5 min at 25°C and the reaction was stopped by adding 0.5 ml of 5% (v/v) sulphuric acid. Enzyme activity was calculated using an extinction coefficient of 25.5 mM^{-1} cm^{-1} at 470 nm for tetra-guaicol.

RESULTS

Phosphoribulo kinase deficient plants (C8) grown at ambient CO_2 level (350 mmol mol^{-1}) showed less growth measured as fresh and dry weight compared to wild type (Bin-19) plants Decrease in the water potential also resulted in further decrease in biomass (Fig. 2A). Well watered (WP= -0.55 Mpa; Fig. 1 and 2A) transformant plants showed 27% less fresh weight as compared to wild type (Bin-19) plants. Water stress to the level of –2.2 Mpa (Fig. 1 and 2A) resulted in 45% decrease in the fresh weight in minus PRK transformant

When plants were grown at higher level of CO_2 (700 mmol mol^{-1}) growth was enhanced significantly in both transformants as well as wild type plants (Fig. 2B). More interestingly even the water stress did not affect the growth significantly compared to plants grown at ambient CO_2 level and exposed to water stress. Even the water stress level of –2.2 Mpa extent, decrease the yield by 27% for wild type and 57% for the transformants as compared to their respective well watered (control) plants. Even the most water stressed (-2.2 Mpa) wild type plants, grown at elevated CO_2, showed 22% higher biomass compared to well watered wild type plants grown at ambient CO_2 level (Fig. 2A & B).

The net CO_2 assimilation was higher in wild type (Bin-19) in well watered (-0.55 Mpa) plants compared to minus PRK (C8) plants irrespective of level of CO_2 (Fig. 3A). However, as the water stress level increased plants (transformants as well as wild type) grown at 700 mmol mol^{-1} CO_2 level showed greater net photosynthesis as compared to plants grown at

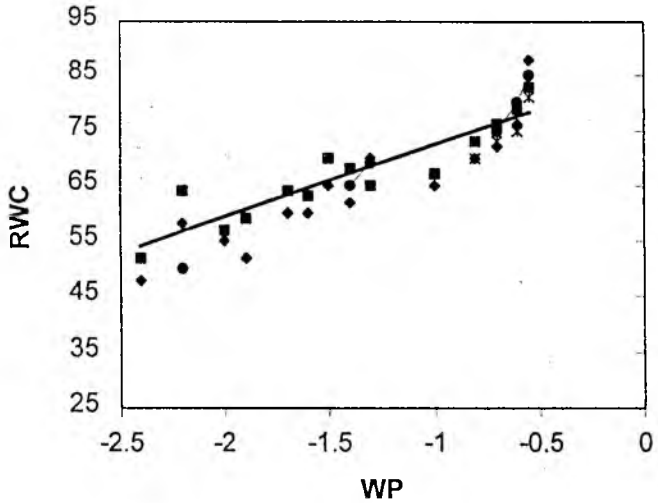

Figure 1 : Relationship between relative water content (RWC) and water potential (Mpa)

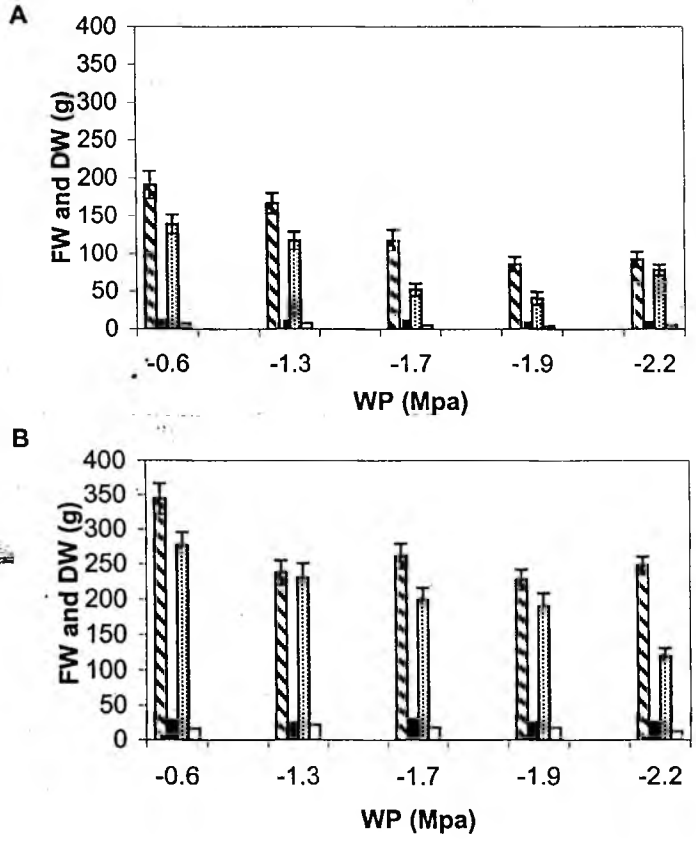

Figure 2 : Effect of 350 µmol mol^{-1} level of CO_2 (A) and 700 µmol mol^{-1} level of CO_2 (B) on fresh weight ▨ and dry weight ■ in wild type and fresh weight ▨ and dry weight □ in transformant plants exposed to different level of water stress. n=5, error bars denote standard deviation.

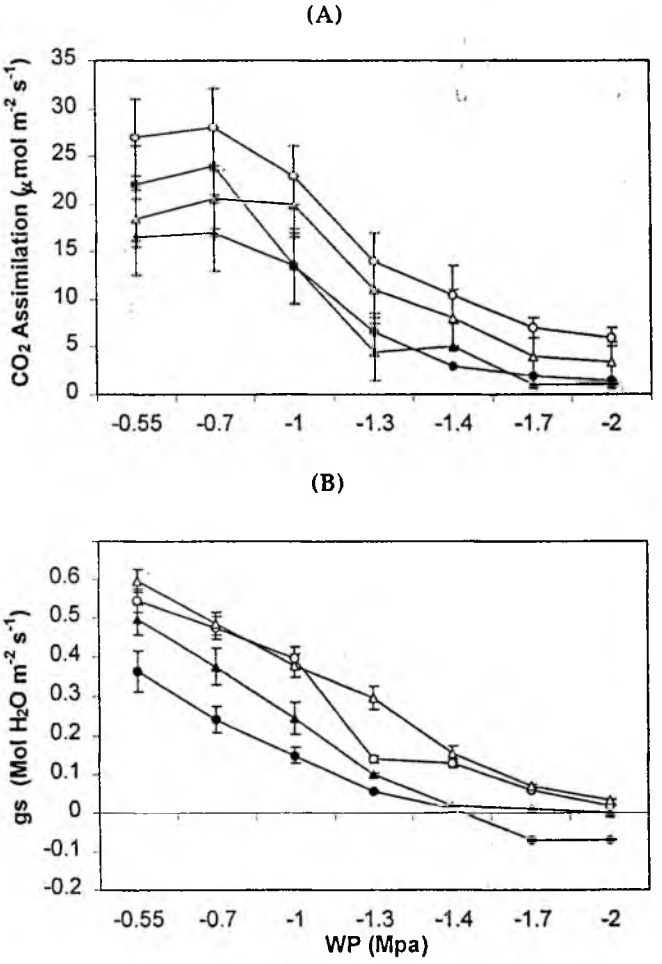

Figure 3 : (A) Net leaf photosynthesis rate (Fc) in 30 days old wild type (—O—; —●—) and transformants (—Δ—; —▲—) tobacco plants grown at 350 µmol mol^{-1} CO_2 (—●—; —▲—) or 700 mmol mol^{-1} CO_2 (—O—; —Δ—) level and exposed to different level of water stress. n=5, vertical bars show standard deviation; (B) Stomatal conductance (gs) in 30 days old wild type (—O—; —●—) and transformants (—Δ—; —▲—) tobacco plants grown at 350 µmol mol^{-1} CO_2 (—●—; —▲—) or 700 µmol mol^{-1} CO_2 (—O—; —Δ—) level and exposed to different level of water stress. n=5, vertical bars denote standard deviation.

350 mmol mol^{-1} CO_2 level. Wild type plants grown at 700 mmol mol^{-1} CO_2 level and water stressed showed slightly higher level of net photosynthesis than transformants plants with respective level of water stress and CO_2.

More or less similar results were observed with regard to stomatal conductance. Both type plants (transgenic and wild) grown at 700 mmol mol^{-1} CO_2 level and experiencing water stress showed relatively less decrease in stomatal conductance then plants grown at 350 mmol mol^{-1} CO_2 level and at respective water stress level (Fig. 3B).

Figure 4 show the changes in chlorophyll fluorescence parameters (Fv/Fm ratio, qP, qN and (PSII) in wild and transformant plants grown at 350 (Fig. 4 A & B) and 700 mmol mol^{-1} CO_2 level (Fig. 4 C & D). Wild type plants grown at ambient CO_2 level (Fig. 4A) showed slight decrease in qP with increasing water stress as compared to minus PRK plants (Fig. 4B) grown at respective water stress level. Similar results were observed when the plants were grown at elevated CO_2 level and exposed to water stress except that qP level was slightly higher than observed under ambient CO_2 level. qN level increased with increasing water stress level in both wild type as well as minus PRK transformant plants

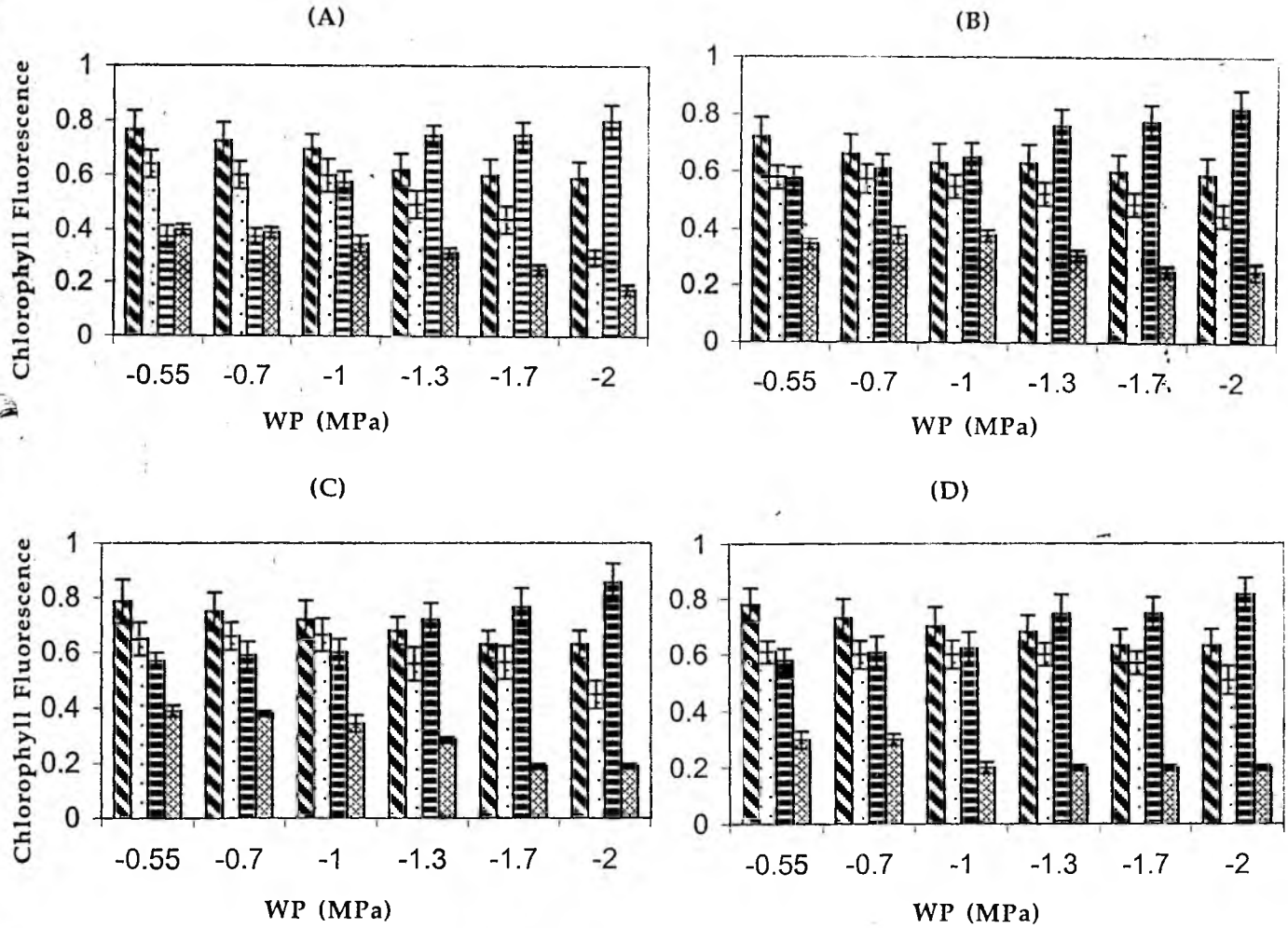

Figure 4 : Fluorescence parameter Fv/Fm ▨, qP ▩, qN ▤ and ϕPS II ▩ as a function of ambient (350 µmol mol^{-1}) CO_2 level (A & B) or elevated (700 µmol mol^{-1}) CO_2 level (C & D) in 30 days old tobacco plants experiencing different level of water stress. n=7, vertical bars denote standard deviation).

grown under ambient or elevated CO_2 level. Interestingly well watered wild type plants grown at ambient CO_2 level showed low level of qN as compared to well watered minus PRK plants. Fv/Fm level was slightly higher in both type of plants grown at 700 mmol mol^{-1} CO_2 levels compared to 350 mmol mol^{-1} CO_2 level. Fv/Fm declined to more or less same level in both wild as well as transformant plants in response to increasing level of water stress in irrespective of CO_2 level. PS II level in well watered wild type plants was slightly higher then seen in well watered minus PRK (transformant) plants. Also the decrease in PSII with increasing level of water stress in minus PRK plants grown at either ambient or elevated CO_2 level was greater compared to observed for wild type plants grown under same conditions.

Peroxidase activity was greater in wild type plants (Bin-19) then observed in transformant plants irrespective of the level of CO_2 (Fig. 6). Plants grown at 700 mmol mol^{-1} level of CO_2 showed far greater activity then seen in plants grown at ambient level of CO_2 and wild type plants showed higher level of peroxidase as compared to minus PRK plants. Well watered plants (wild as well as transformant) grown at ambient level of CO_2 showed far less increase in the peroxidase level then seen in plants grown at elevated CO_2 level. Increasing water stress, in both type of plants resulted in steep increase in the peroxidase level irrespective of CO_2 level (Fig. 6).

Levels of all xanthophylls and β-carotene was greater in wild type plants than in minus PRK plants (Fig. 5). Zeaxanthin increased to more or less same extent with increasing water stress in both wild as well as transformant plants. Decrease in violaxanthin was more pronounced with increasing level of water stress in wild type plants then seen in transformat

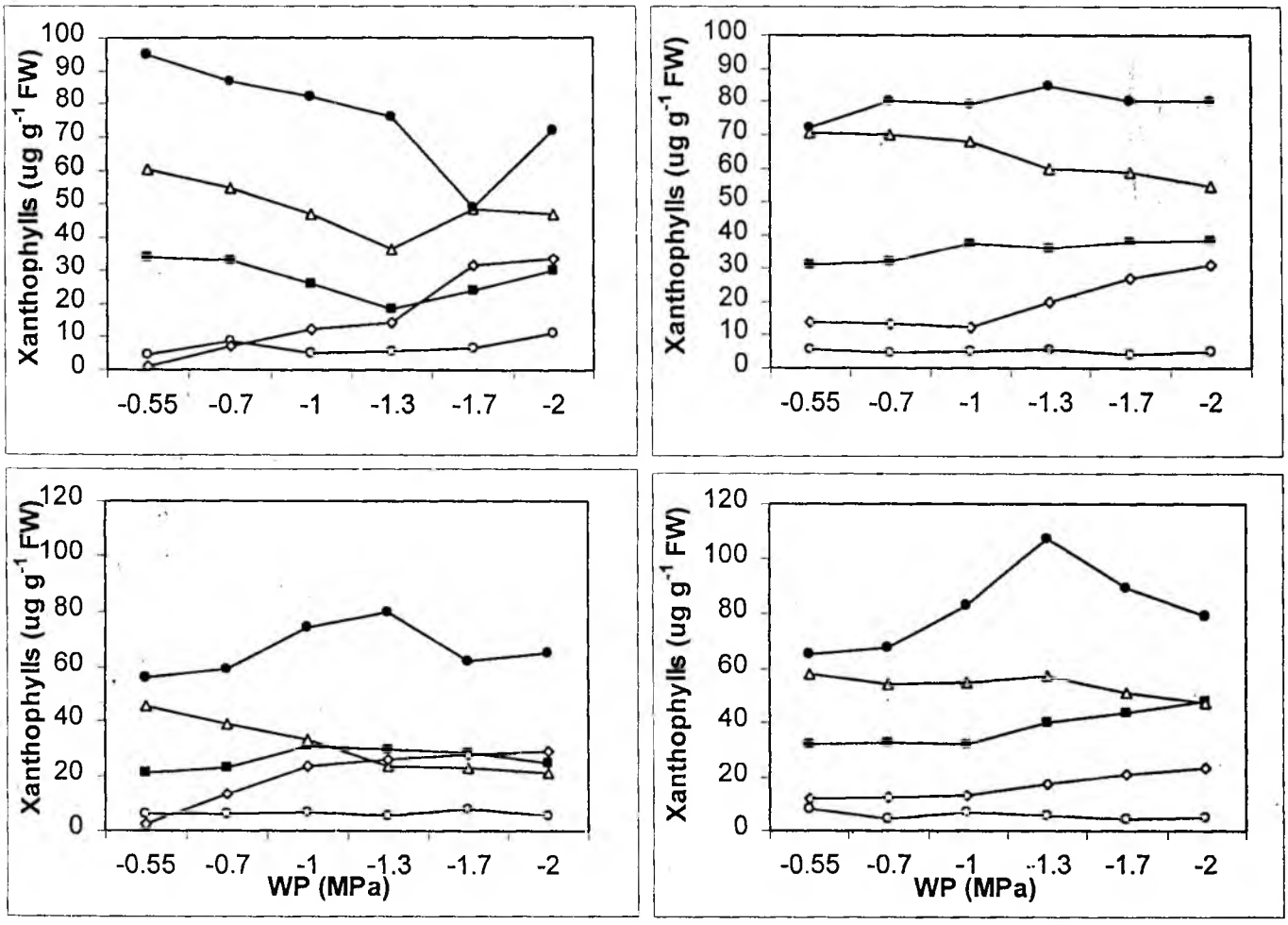

Figure 5 : Pigment content β-carotene (—●—), violaxanthin (—Δ—), neoxanthin (—■—), antheraxanthin (—○—) and zeaxanthin (—◇—) in wild type (A and C) and transformant (B and D) tobacco plants grown at ambient (350 μmol mol^{-1}) CO_2 level (A and B) and elevated (700 μmol mol^{-1}) CO_2 level and exposed to different level of water stress. n=4, vertical bars show standard deviation.

transformant plants when water stressed whereas it decreased in wild type plants in response to water stress under ambient CO_2 condition (Fig. 5 A and B). Similarly b-carotene level in wild type plants decreased with increasing level of water stress (Fig. 5A) while b-carotene content increased in transformant plants when exposed to increasing level of water stress (Fig. 5B).

Plants grown at 700 mmol mol^{-1} CO_2 (wild as well as transformant; Fig. 5 C & D) and fully watered showed more or less same level of xanthophylls as seen in plants grown at 350 mmol mol^{-1} CO_2 level. However, changes in the xanthophylls with respect to increasing level of water stress was less in plants grown at elevated CO_2 than at ambient CO_2 level. Wild type plants grown at elevated CO_2 showed greater decrease in violaxanthin and increase in zeaxanthin with regard to increasing level of water stress (Fig. 5C) then was observed in transformant plants for respective level of water stress (Fig. 5D). Slight increase in neoxanthin in wild as well as transformed plants grown at elevated CO_2 was observed with increasing level of water stress.

There was very little difference in ABA content of plants whether grown at ambient or elevated CO_2. Also wild type as well as transformed tobacco plants did not show great deal of differences in their ABA content. However, ABA content in both, transformed as well as wild type plants increased more than three folds with increasing level of water stress (Fig. 7).

DISCUSSION

Our results of increased biomass production under CO_2 enrichment are in consonant with existing work in the field. Idso *et al.* (2000) reported increase in under ground as well as above ground biomass in *Hynenocallis littoralis* when grown at 700 mmol

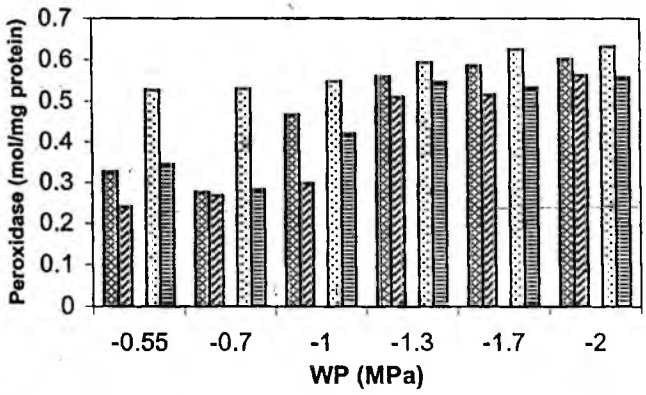

Figure 6 : Ascorbate peroxidase content in 30 days old wild type (▩ , ▦) and transformant (▨, ▤) tobacco plants grown at ambient (350 µmol mol^{-1}) CO_2 (▩ , ▨) or elevated (700 µmol mol^{-1}) CO_2 (▦ , ▤) and exposed to different level of water stress. n=3, vertical bars denote standard deviation.

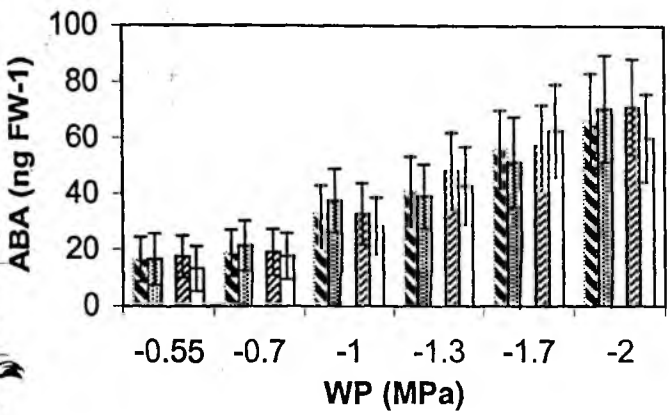

Figure 7 : Effect of ambient (▧ , ▦) or elevated (▨ , □) CO_2 level on ABA content in 30 days old wild type (▧ , ▨) and transformant (▦ , □) tobacco plants exposed to different level of water stress. n= 3, vertical bars denote standard deviation.

mol^{-1} CO_2. Similarly Drake *et al.* (1997) showed increased resource use efficiency, reduced stomatal conductance and transpiration and improve water use efficiency and light use efficiency when grown at elevated CO_2 level. Woodrow (1994) working with various C3 plants showed that there is a change in both the carbon flux and the pattern of nitrogen allocation when plants are grown under elevated CO_2. Baker *et al.* (1997a) reported that rice seedling grown under elevated CO_2 showed a modest reduction of 10% in water use efficiency to increase drought tolerance and showed that this water [illegible]

under elevated CO_2 allowed photosynthesis to continue at normal rate for about two more days under drought conditions. Similar results of elevated CO_2 promoting growth was reported by many other authors (Wilson and Bunce, 1997; Mandesscheid *et al.*, 2003; Holtum and Winter, 2001; Ziska and Bunce, 1999; Ziska and Teasdale, 2000 and Barrett and Gifford, 1995). However, Sarlabai *et al.* (1997) reported no great advantage in C3 and C4 plants grown under elevated CO_2. They reported inactivation of RUBISCO and lowering of stomatal conductance under CO_2 enrichment.

The decrease in net assimilation rate of CO_2 in transformant tobacco plants with only 5% of wild type PRK is due to the lower enzyme concentration which results in over 2-fold drop in RUBP concentration and thus present a major limitation to photosynthesis (Paul *et al.*, 1995). The wild type plants (Bin-19) responded to the increase level of CO_2 where as minus PRK plants (C8) did not show any increase in fresh and dry weight as a result of elevated CO_2 (Fig. 2 A & B). This could be due to wild type plants being able to fix CO_2 at a higher rate while transformant plants could not fix the CO_2 inspite of elevated level due to the limitation of the substrate RUBP as a result of limited availability of Phosphoribulokinase (only 5% of wild plants). Woodrow (1994) reported that by doubling of atmospheric CO_2, the activity of RUBISCO was increased and therefore change in the rate of CO_2 assimilation would largely depend on the concentration of RUBP rather than the activity of Rubisco in this study. Though the increase in biomass in the transformant plants under water stress condition in enriched CO_2 environment suggest that RUBP limitation might have been over come to some extent in these plants probably by the oxidative pentose phospate pathway or the increase in biomass could also be due to lower rate of photorespiration under elevated level of CO_2.

Wild type as well as transformant plants grown at elevated CO_2 efficiently mitigated severe water stress condition compared to their counterparts grown at ambient CO_2, indicating better water use efficiency at elevated CO_2 level (Fig. 2 A & B). Both CO_2 assimilation (FC) and stomatal conductance (gs) also increased even under water stress conditions when grown at elevated CO_2 level. Rogers *et al.* (1984), Baker *et al.* (1997b), have shown that CO_2 enriched plant grown under draught stress have increased growth compared to plants grown at ambient CO_2 level. They reported that CO_2 enrichment increased stomatal conductance to maintain high level of leaf water potential to avoid loss of turgor (drought) and its consequences to leaf

better water use efficiency in plants grown at elevated CO_2. Our results also indicate similar observation, probably by maintaining higher water potential (higher level of gs) thus mitigating the effect of water stress in wild as well as transformant plants at elevated CO_2 level.

The reason for much higher increase in the biomass as compared to net photosynthesis in wild as well as transformant plants under enriched CO_2 and water stress condition in our study might also be due to the result of that elevated concentration of CO_2 in the dark have also been shown to decrease CO_2 efflux in several species (Bunce, 1995; El-Kohen *et al.*, 1991). The resulting conversion of carbon has been shown to increase the dry mass of alfalfa exposed to elevated CO_2 (Reuveni and Gale, 1985). Mechanism by which elevated CO_2 concentration might reduce CO_2 efflux in the dark include a stimulation of dark CO_2 fixation and reduction in flow through the rapid reversible decrease in respiration (Bunce, 1995). Leaf area increase as well as increase rate of photosynthetic under elevated level of CO_2 conditions may also account for increased fresh and dry weight in our study. In this study better Pn even at high water stress conditions (-2.0 Mpa) may largely be due to maintaining higher stomatal conductance as compared to ambinet CO_2 grown plants which would also lower the rate of photorespiration as a result of higher stomatal conductance as compared to observed at ambient CO_2 levels resulting in greater productivity (fresh and dry weight). However, higher stomatal conductance should result in more rapid soil drying and thereby putting itself at greater risk if drought condition are prolonged. Similar result were also reported by Heath and Kersteins (1997) in Beech plants. Idso *et al.* (2000) had reported approximately 56% increase in the below ground biomass under elevated CO_2 level. This is possible that this increase in the below ground biomass may also explain better growth under drought conditions in CO_2 enriched plants inspite of higher stomatal conductance in our study as root will be able to reach deeper in the soil for water. However, we have not measured the below ground biomass.

The results with Fv/Fm ratio, qP, qN and PSII suggest that neither the CO_2 enrichment nor the water stress level made any significant effect on parameters indicative of various state of light reaction of photosynthesis, indicating that neither the enrichment of CO_2 nor the water stress significantly affected the light reaction in either wild or transformant plants. The results can be explained on the basis of that transformant plants lack phosphoribulokinase an enzyme which is to synthesize RUBP from R5P using an ATP to be used as a substrate for CO_2 fixation and may not directly influenced the photosynthetic electron transport chain (light reaction) significantly. Earlier work by Sharma and Singhal (1993) had also shown that water stress as such do not cause damage to electron transport chain. Ogaya and Penuelas (2003) have shown in *Quercus ilex* and *Phillyrea latifolia* that 15% drop in the normal water level did not significantly change the Fv/Fm value nor the photosynthetic electron transport unless the drought was combined with the cold. Similarly Chen *et al.* (2004) in *Rumex* leaves under salt stress condition, which also causes osmotic stress, did not report any decrease in the fPSII or Fv/Fm ratio.

The results with peroxidase activity indicate no oxidative stress as a result of CO_2 enrichment, actually our results on Pn also showed that CO_2 enrichment resulted in better water use efficiency and to a large extent mitigated the effect of water stress. It is probable that it might be related to better managing the osmotic stress by managing the internal plant water relation, probably by changing in the solute potential rather than the turgor potential. Also better protection of wild as well as transformant plants under elevated CO_2 conditions could also be due to doubling of peroxidase there by quenching the free radicals more efficiently and resulting in lesser damage and thus higher Pn (Fig. 3).

CO_2 enrichment also did not change carotenoid levels significantly. Though violaxanthin (V) to zeaxanthin (Z) was better in wild type plants than transformant plants. Probably as a result of less epoxidation of V to Z in transformant plants were somewhat limited in their ability to dissipate the excess light energy as compared to wild type plants. Xanthophylls are reported to have role in energy dissipation (Sharma, 2002; Demmig-Adams, 1990). In this study it is possible that transformant plants will suffer due to over energization as a consequence of substrate limitation for CO_2 fixation. Transformant plants showing less xanthophyll and pigment content (compared to wild type) may play a role in preventing over energization by decreasing the amount of light being absorbed. However, xanthophyll cycle was also operating less efficiently in transformant plants exposed to water stress than wild type plants, therefore, more damage to transformant plants. This could also be one of the reason for greater damage to photosynthesis in minus PRK plants under water stress conditions, since water stress will predispose plants to over energization . even under low light.

As expected with increasing water stress there was a tremendous increase in the ABA content.

However, neither the CO_2 enrichment nor the modification of metabolic path way in transformant plants showed any appreciable influence on ABA accumulation. Our results indicate that the accumulation of ABA in our study was solely due to water stress.

Parry *et al.* (1992) have reported the synthesis of ABA in root under water stress condition. Werner *et al.* (1991) reported that ABA may provide tolerance against drought stress, not necessarily by reducing water loss due to stomatal regulation but by induction of synthesis of new protein induced by ABA.

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