Hormonal regulation of photosynthetic enzymes in cotton under water stress

D.M. PANDEY, C.L. GOSWAMI, B. KUMAR, and Sudha JAIN

Department of Botany and Plant Physiology, CCS HAU Hisar-125 004, India

Abstract

Activities of ribulose bisphosphate carboxylase/oxygenase (RuBPCO), phosphoenolpyruvate carboxylase (PEPC), and carbonic anhydrase (CA) were determined in leaves of cotton (*Gossypium hirsutum* L. cv. H-777) subjected to 8-d waterlogging (WL) at the vegetative stage, or to drought (D) at the reproductive stage, or to interaction of both stresses. The soil moisture of control plants was kept at field capacity. One day prior to stress various growth hormones (5 μM) were sprayed up to runoff. WL reduced RuBPCO and CA activities, while PEPC activity increased. Upon D, RuBPCO and PEPC activities were reduced while CA activity was increased. Imposition of both stresses increased activities of all three enzymes. Effect of stresses on enzyme activity was alleviated by benzylaminopurine (BAP), but indol-3-yl-acetic acid was more promoting under interactive stress. No CA activity with BAP was observed during interactive stress.

Additional key words: abscisic acid; benzylaminopurine; carbonic anhydrase; ethrel; gibberellic acid; *Gossypium hirsutum*; phosphoenolpyruvate carboxylase; ribulose-1,5-bisphosphate carboxylase/oxygenase.

Introduction

The ability to maintain the rate of photosynthetic carbon dioxide fixation and nitrate assimilation under environmental stresses is fundamental to the maintenance of plant growth and production (Lawlor and Uprety 1993). Under water stress (WL or D), endogenous contents of auxins, gibberellins, and cytokinins decrease while contents of abscisic acid and ethylene increase (Nilsen and Orcutte 1996). This effect of water stress is regulated either by affecting synthesis, degradation, or transport of hormones. In addition, sensitivity of the tissue can also be changed (Davies 1995).

Under both WL and D, RuBPCO activity becomes inhibited (Bradford 1982, Berkowitz and Gibbs 1983, Berkowitz and Whalen 1985). In severely dehydrated leaves RuBPCO activity is considerably decreased (Kicheva *et al.* 1994). On the contrary, PEPC activity in maize increased slightly during water deficit (Foyer *et al.* 1998) while in wheat PEPC activity was not altered (Kicheva and Lazova 1997). Carbonic anhydrase activity at mild water stress (RWC 81 %) is decreased but at severe water stress (RWC 74 %) it is increased (Kicheva and Lazova 1997).

Application of GA_3 to red clover increased RuBPCO activity and thus under normal condition improved photosynthesis (Treharne and Stoddart 1968). Short-term treatment with ABA decreased stomatal conductance, and net photosynthetic rate (P_N), and increased CA activity in 10-d-old pea seedlings (Lazova *et al.* 1999). Cytokinins induced synthesis of important proteins that increased plant resistance to various stressors including water deficit (Kulaeva 1982, Kislyakova *et al.* 1989). Spraying of plant with cytokinin 4-PU-30 before and after water stress alleviated the negative effect of water stress on lipid membrane composition permitting the plants to resist harmful environment (Ivanova *et al.* 1998). The present study was, therefore, conducted to alleviate the deleterious effects of water stress on the activity of photosynthetic enzymes by plant growth hormones.

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E-mail: hau@hau.hry.nic.in
Abbreviations: ABA - abscisic acid; BAP – benzylaminopurine; CA - carbonic anhydrase; D – drought; DTT – dithiothreitol; Eth – ethrel; EDTA – ethylenediamine tetraacetic acid; FC - field capacity; GA_3 - gibberellic acid; IAA – indole-3-yl-acetic acid; MCE – mercaptoethanol; PEPC - phosphoenolpyruvate carboxylase; PVP - polyvinyl pyrrolidone; RuBPCO – ribulose-1,5-bisphosphate carboxylase/oxygenase; RWC – relative water content; WL - waterlogging.
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Materials and methods

Plants: Acid de-linted seeds of American cotton (*Gossypium hirsutum* L.) cv. H-777, a widely grown cultivar in Haryana, were sown in polythene bags filled with 10 kg thoroughly washed dune sand. Ten days after sowing, 100 cm^3 of complete Hoagland solution (Hoagland and Arnon 1950) was added to each bag and the plants were thinned. Further, 150 cm^3 Hoagland solution supplemented with 40 mM KNO_3 was applied to each bag at 10-d interval.

Stress treatment: The cotton plants were grouped in four sets. The first set of plants was subjected to 8 d of WL at vegetative stage (*i.e.*, after 35-40 d of sowing) by placing the bags in plastic buckets so that water always remained 2 cm above the sand surface. In the second set of plants, at reproductive stage (*i.e.*, 55-60 d of sowing), watering was withhold till the plants attained permanent wilting percentage (soil moisture content decreased to 4.5 %). In the third set of plants, WL at vegetative stage was followed by D at reproductive stage. The soil moisture content in fourth set of plants was kept at field capacity (27 %) and these served as control. IAA, GA_3, BAP, ABA, and ethrel at 5 μM concentration were sprayed to run off, one day prior to stress treatment.

Extraction of enzymes: At the end of stress treatment, fully expanded leaves (3-5 from the top) were frozen in liquid nitrogen and kept at –80 °C. The leaf tissue was extracted by grinding in a chilled mortar and pestle with 2.5 cm^3 of Tris-HCl buffer (0.05 M, pH 7.4) that contained 0.4 μM PVP, 1 mM DTT, 2 mM EDTA, 5 mM MCE, 0.01 % *Triton-100 X*, 20 mM $MgCl_2$, and 5 mM $NaHCO_3$. The extract was centrifuged at 12 000×*g* for 30 min at 0-4 °C. The supernatant was collected and filled up to 3.0 cm^3 with extraction buffer. This is referred as enzyme extract and was used for further analysis of enzyme activity. The activities of various enzymes were observed during the linear time coarse of the assays.

Enzyme assays: RuBPCO (EC 4.1.1.39) activity was estimated by a radiochemical method of Björkman (1968). To 0.1 cm^3 of enzyme extract in a scintillation vial 1.0 cm^3 of reaction mixture containing CO_2-free 0.05 mM Tris-HCl buffer, pH 8.2, 40 mM $MgCl_2$, and 100 mM $NaHCO_3$, 0.37 MBq $NaH^{14}CO_3$ (spec. activity 1.89 kBq mol^{-1}), and 81 mM DTT was added. Then 0.25 cm^3 of RuBP (0.4 mM) was added to the vial. The reaction was allowed to proceed at 30±2 °C for 1 min and terminated by the addition of 0.2 cm^3 of 2 M HCl. The sample was heat-dried and reconstituted with 0.2 cm^3 of 2 M HCl, again heat-dried and dissolved in 0.5 cm^3 of distilled water. Then 4.5 cm^3 of liquid scintillation fluid (Brey 1960) was added to each scintillation vial and radioactivity counted with a *LKB WALLAC, 1209 RACKBETA*, Liquid Scintillation Counter.

PEPC (EC 4.1.1.31) was estimated by a radiochemical method of Ashton *et al.* (1990). To 0.1 cm^3 of enzyme extract in a scintillation vial 0.3 cm^3 reaction mixture was added, containing 0.05 mM Tris-HCl buffer, pH 8.2, 40 mM $MgCl_2$, 100 mM $NaHCO_3$, 20 mM PEP, and 81 mM DTT. After 10 min of incubation at 30±2 °C the enzyme reaction was terminated by the addition of 0.2 cm^3 of 2 M HCl. The samples were processed as for RuBPCO.

CA (EC 4.2.1.1) activity was estimated by the method of Chang (1975) using a Wilson respirometer. An aliquot of 0.5 cm^3 (0.05 M) $NaHCO_3$ and 3.0 cm^3 phosphate buffer (0.05 M, pH 7.0) were taken in main compartment of a Warburg flask and 0.5 cm^3 enzyme extract was kept in side arm. After equilibrium two solutions were mixed and shaken at 2.18 oscillations per s and changes in pressure were recorded. CA from *Sigma* was used as standard, one unit of which produced 0.22 μm^3 change in pressure per s.

Results and discussion

In control plants all hormones enhanced RuBPCO activity and IAA was most stimulatory. WL and D reduced RuBPCO activity, while both stresses increased it significantly. BAP increased RuBPCO activity under WL as well as under D. IAA was most promoting during WL+D (Fig. 1). In severely dehydrated leaves RuBPCO activity is considerably decreased (Kicheva *et al.* 1994) which supports our findings. Drought-induced decrease in RuBPCO activity should be attributed not only to proteolytic decomposition of enzyme protein but also to partial inhibition of its catalytic activity because the decrease in RuBPCO activity was more than that in RuBPCO content (Chernyad'ev and Monakhova 1998). The literature contains conflicting opinions of water stress effect on RuBPCO. The activity of it under D either remained unchanged or increased, slightly decreased, even fell sharply depending upon the intensity of water stress and plant type (Chernyad'ev 1997). The loss of large (55 kDa) and small (15 kDa) subunits of RuBPCO was found in water stressed seedlings of *Erythrina variegata* (Muthuchelian *et al.* 1997). BAP or 6-furfurylaminopurine affected photochemical activity (Doushkova *et al.* 1989, Muthuchelian *et al.* 1994) and the carboxylating activity and content of RuBPCO (Lerbs

et al. 1984, Chernyad'ev 1994). Kartolins affect the activities of RuBPCO and other photosynthetic enzymes and ultrastructure of leaf plastids in crop plants exposed to drought (Kulaeva 1985, Baskakov 1988, Kislyakova *et al.* 1989), and thus increase resistance to drought.

In control plants, PEPC activity was maximum reduced by IAA. PEPC activity increased with WL and WL+D while it decreased with D. Increased PEPC activity under water stress may contribute towards net photosynthetic rate (P_N) in a similar way as suggested by Fontaine *et al.* (1999) in *Pinus halepensis* exposed to ozone. Contrary to our findings, in severely dehydrated leaves of wheat PEPC activity is not altered (Kicheva and Lazova 1997). Upon WL, PEPC activity decreased maximum with ABA. Under D, all hormones increased PEPC activity. Eth and BAP had maximum effect. IAA was most promotory during WL+D (Fig. 1). The promoting effect of BAP and 4-PU-30 on photochemical activity and on the number of oxygen evolving centres under water stress is thus probably a consequence of certain changes in the chloroplast structure and oxygen evolving enzyme system of photosynthetic apparatus (Metwally *et al.* 1997).

CA activity in the control was reduced by various

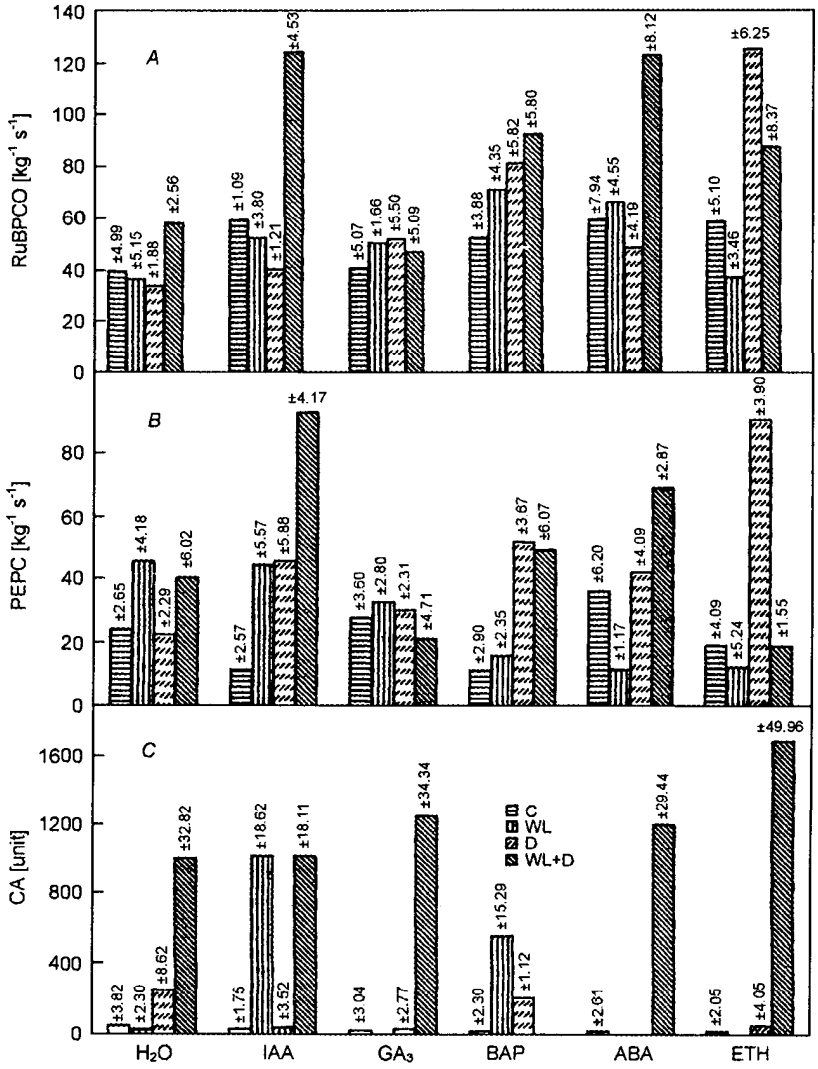

Fig. 1. Effect of growth regulators on activities of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO, *A*), phosphoenolpyruvate carboxylase (PEPC, *B*), and carbonic anhydrase (CA, *C*) in cotton leaf under different stresses. Plants were sprayed with various hormones at 5 μM concentration before imposing the stress.

growth hormones. However, in contrast to our findings, short-term treatment with 10 μM ABA increased CA by 73 per cent (Lazova *et al.* 1999). WL reduced CA activity while during D and WL+D the CA activity increased many fold. During WL, IAA and BAP were more promotory on CA activity, while no detectable activity was seen with ABA under D and with BAP during WL+D (Fig. 1). Besides stomatal limitation a decreased

mesophyll conductance to CO_2 transfer may limit chloroplast CO_2 and thus photosynthesis when mild leaf dehydration is manifested (Renou *et al.* 1990, Caemmerer and Evans 1991). Since CO_2 transfer conductance depends on CA activity (Makino *et al.* 1992), this activity might become limiting to photosynthesis under water deficit. During rehydration exogenous application of cytokinin may promote the plant recovery (Metwally *et al.* 1997).

We conclude that in control plants the increase in RuBPCO activity was induced with IAA and ABA, and PEPC activity was increased maximum by ABA. However, CA activity decreased with all hormones. During WL, RuBPCO activity decreased while PEPC increased under WL and WL+D. CA activity increased with D and WL+D. Maximum increase in RuBPCO and PEPC activities was obtained during WL+D by IAA and ABA. During D, Eth gave maximum RuBPCO and PEPC activities. During WL, GA_3, ABA, and Eth completely inhibited CA activity. Maximum increase in CA activity during WL+D was obtained by Eth, however, BAP completely suppressed CA activity during WL+D.

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