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BRIEF COMMUNICATION

Light induced enhancement in proline levels under stress is regulated by non-photosynthetic events

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Abstract

Vigna radiata (L.) seedlings (5-d-old) were exposed to different concentrations of NaCl in light and in dark. The content of proline in the shoots increased with an increase in NaCl concentration, in light as well as in dark. But, irrespective of the concentration of NaCl, proline accumulation in the shoots was higher in light than in dark. Pretreatment of seedlings with dichlorophenyl dimethyl urea (DCMU) did not make any significant difference in light promoted stress induced proline accumulation. As DCMU is a potent inhibitor of photosynthetic electron transport, the light reaction of photosynthesis was not responsible for the observed light promotion of stress induced proline accumulation. In another set of experiments, 5-d-old green as well as etiolated seedlings were exposed to NaCl stress in the presence of different concentrations of sucrose. Irrespective of the concentration of sucrose used, proline content in shoots of stressed seedlings was higher in light than in dark. Although, sucrose enhanced NaCl stress induced increase in proline content in dark by about 32 %, this enhancement was not comparable to the 286 % increase in proline content brought about by light. These results showed that certain factors other than photosynthesis play a role in light promotion of stress induced proline accumulation.

Additional key words: DCMU, etiolated plants, NaCl, photosynthesis, sucrose, *Vigna radiata*.

In nature, plants are exposed to a variety of environmental stresses and the plants respond to these stresses by altering some of their physiological and biochemical processes. One of the most frequently observed responses of plants exposed to various environmental stresses such as drought, salinity, light, temperature and heavy metal stress (Aspinall and Paleg 1981, Nikolopolus and Manetas 1991, Alia and Pardha Saradhi 1991, 1993, Delauney and Verma 1993, Pardha Saradhi *et al.* 1995, Hong *et al.* 2000, Mansour 2000, Dash and Panda 2001 and references therein) is the accumulation of proline.

Light has been shown to promote stress induced proline accumulation (Arora and Pardha Saradhi 1995, Fedina and Popova 1996 and references therein). Although it has been suggested that the light stimulated proline accumulation is dependent upon photosynthesis (Noguchi *et al.* 1966, Stewart *et al.* 1966, Greenway and Setter 1979, Pesci 1993), the response has not been examined in detail. Understandably, the increased polysaccharide pool resulting from photosynthesis may contribute towards synthesis of proline and its precursors (Stewart 1978). However, our earlier experiments carried out with plants and plant parts devoid of complete photosynthetic apparatus indicated that enhancement in the stress induced proline accumulation in light is mediated through processes other than photosynthesis (Arora and Pardha Saradhi 1995). Joyce *et al.* (1984) observed acceleration of proline accumulation by very low irradiances that were not sufficient for net CO_2 assimilation. In the light of the above information, present experiments were carried out to study the role of photosynthesis in light promoted stress induced proline accumulation.

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Abbreviation: DCMU - [(3,4-dichlorophenyl)-1,1-dimethylurea].

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Seeds of *Vigna radiata* (L.) Wilczek cv. Pusa-105 were obtained from Indian Agricultural Research Institute, New Delhi. Seeds were raised on mineral growth medium as described earlier (Arora and Pardha Saradhi 1995).

Five-day-old seedlings grown in growth chambers, with a 16-h photoperiod (photon flux density 120 μmol m^{-2} s^{-1}) and temperature at 25 ± 1 °C, were exposed to salt stress by transferring them to mineral growth medium supplemented with different concentrations of NaCl (0, 50, 100, 150, 200 and 250 mM). One set of unstressed and stressed seedlings was kept in light (16-h photoperiod, irradiance 120 μmol m^{-2} s^{-1}) and the other similar set was kept in continuous darkness. Proline content was determined in the shoots of seedlings 3-d after exposing them to NaCl-stress.

To determine the involvement of photosynthesis in light stimulated enhancement in salt-stress induced proline accumulation, two types of experiments were carried out. In the first type, 5-d-old seedlings were sprayed with 100 mM (3,4-dichlorophenyl)-1,1-dimethylurea (DCMU). Fifty per cent of the seedlings were exposed to NaCl stress and the rest served as NaCl-unstressed seedlings. In the second type of experiments mineral growth medium was supplemented with 0, 1, 2, and 4 % (m/v) sucrose, at the time of exposure of 5-d-old green (light grown) and etiolated (dark grown) seedlings to NaCl stressed and unstressed conditions. Half of the seedlings from all the above treatments were exposed to 16-h photoperiod and the other half was kept in continuous darkness. The amount of proline in the shoots of seedlings was determined 3 d after exposure to various treatments.

For proline estimation, the plant material was crushed in 3 % sulphosalicylic acid and the homogenate was centrifuged at 15 000 *g* for 15 min at room temperature and the supernatant was used for proline quantification by the method of Bates *et al.* (1973). Concentration of proline in the sample was computed using a standard of L-proline. All the experiments were carried out at least three times.

Growth of 5-d-old seedlings, measured in terms of shoot length, fresh mass and dry mass, exposed to NaCl (even at concentrations as low as 50 mM) was significantly lower than that of the control seedlings (data not shown). Proline content in the shoots increased with an increase in the concentration of NaCl in the growth medium, both in light as well as in dark (Fig. 1). However, the enhancement in proline content was remarkably higher in light than in dark (*e.g.* at 200 mM NaCl the enhancement was 10 and 2.2 fold over unstressed seedlings in light and dark, respectively).

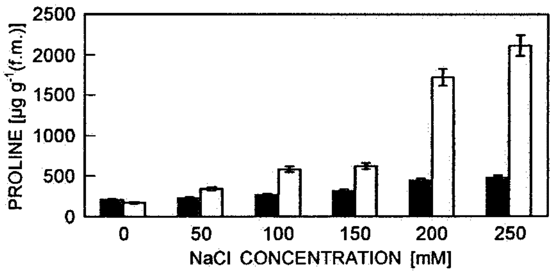

Fig. 1. Proline content in the shoots of *Vigna radiata* seedlings exposed to mineral growth medium supplemented with different concentrations of NaCl, in dark (*black columns*) and light (*white columns*). Mean of at least three independent experiments, each with two replicates ± SE.

In order to ascertain the probable link between photosynthesis and accumulation of proline under salinity stress, we used DCMU (a well known inhibitor that suppresses the photochemical electron transport from Q_A to Q_B). DCMU treatment to 5-d-old seedlings in the presence as well as absence of NaCl led to a considerable decrease in the growth of seedlings, measured in terms of fresh mass of the shoots (Fig. 2*A*). DCMU treatment also induced necrosis at the tips of primary leaves but only of seedlings kept in light.

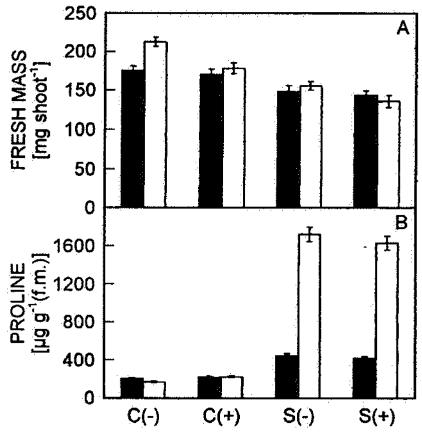

Fig. 2. Fresh mass (*A*) and proline content (*B*) of the shoots of *Vigna radiata* seedlings treated without (-) and with (+) DCMU in absence (C) and presence (S) of 200 mM NaCl in dark (*black columns*) and light (*white columns*). Mean of at least three independent experiments, each with two replicates ± SE.

Seedlings exposed to DCMU treatment for a period of 3-d showed only minor alterations in their proline content and the extent of enhancement in stress-induced proline accumulation by light did not differ significantly (Fig. 2*B*). Under NaCl stress, DCMU-treated and untreated seedlings showed 293 and 286 % higher contents of proline in light than in dark (Fig. 2*B*). As DCMU treatment could not cause any reduction in stress-induced proline accumulation, in shoots of seedlings kept in light, to the level similar to that observed in dark, it can be concluded that the light-promoted stress-induced proline accumulation is not directly linked to the photosynthetic light reactions.

In order to ascertain if photosynthate has any role in stress-induced proline accumulation, experiments were carried out to study the effect of exogenous application of sucrose (a major translocatable sugar in plants) on proline accumulation. 5-d-old light grown seedlings further exposed to light, both in the presence and absence of NaCl, showed lesser increase in shoot fresh mass in the presence of sucrose than in its absence (Fig. 3*A*). The extent of reduction in the fresh mass, increased with increase in the concentration of sucrose. In contrast, the presence of 1 % sucrose significantly increased fresh mass of the seedlings kept in dark, both in the presence and absence of NaCl (Fig. 3*A*). However, 4 % sucrose suppressed the growth of seedlings both in the presence and absence of NaCl.

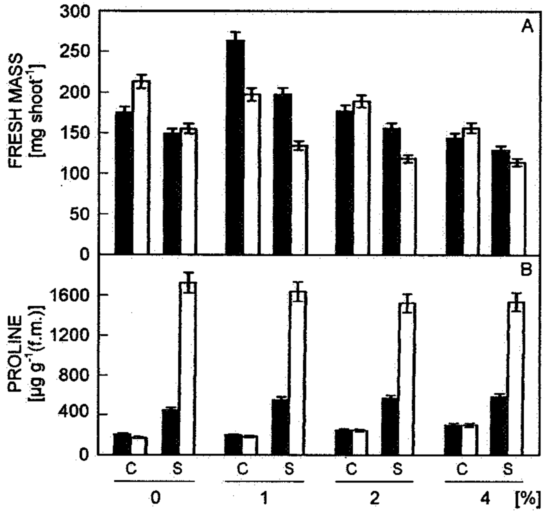

Fig. 3. Fresh mass (*A*) and proline content (*B*) of the shoots of light grown *Vigna radiata* seedlings exposed to mineral growth medium supplemented with different concentrations of sucrose, in absence (C) and presence (S) of 200 mM NaCl in dark (*black columns*) and light (*white columns*). Mean of at least three independent experiments, each with two replicates ± SE.

In 5-day-old light grown seedlings exposed to sucrose under 0 mM NaCl, proline content increased significantly both in light as well as in dark and the extent of increase in proline content increased with increase in concentration of sucrose (Fig. 3*B*). On the other hand, under NaCl-stress the proline content in the shoots of seedlings kept in light decreased by 5 to 7 % with increase in concentration of sucrose. However, proline content in the shoots of stressed seedlings kept in dark, increased with an increase in the concentration of sucrose but the percentage increase in proline contents varied (from 2 to 23 %). Interestingly, the content of proline in seedlings under NaCl-stress was significantly higher in light than in dark, irrespective of the concentration of sucrose (Fig. 3*B*).

The effect of sucrose was also tested on etiolated seedlings. In general, sucrose (at and above 2 %) lowered shoot growth (fresh mass) of the seedlings in dark, at all the NaCl concentrations (Fig. 4*A*). However, only marginal alteration in the shoot growth was recorded in the seedlings exposed to light (Fig. 4*A*).

In contrast to a significant enhancement in the content of proline in light grown seedlings exposed to increasing concentration of sucrose, the proline content in etiolated seedlings exposed to increasing concentration of sucrose in dark did not show any significant difference. Interestingly, under 0 mM NaCl etiolated seedlings exposed to light showed a significant decline in the content of proline in presence of sucrose at and above 2 % (Fig. 4*B*).

Irrespective of the concentration of sucrose, the extent of NaCl-stress induced proline accumulation in both light grown as well as etiolated seedlings was significantly higher in light than in dark. In other words, sucrose in no way could replace light promoted NaCl-stress induced proline accumulation. These results strongly indicate that light promoted stress induced proline accumulation is not linked directly to photosynthesis and suggest the probable involvement of some photoreceptors.

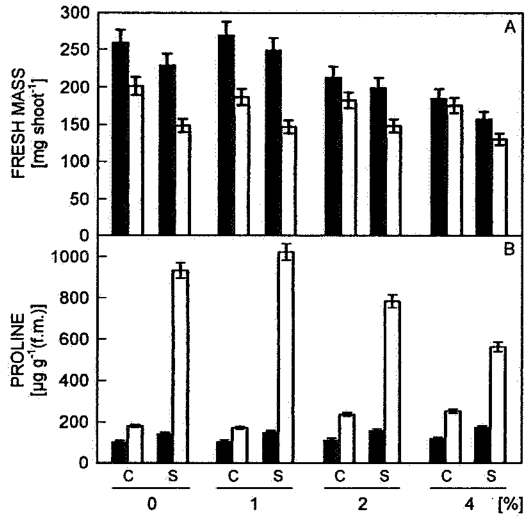

Fig. 4. Fresh mass (*A*) and proline content (*B*) of the shoots of etiolated *Vigna radiata* seedlings exposed to mineral growth medium supplemented with different concentrations of sucrose, in absence (C) and presence (S) of 200 mM NaCl in dark (*black columns*) and light (*white columns*). Mean of at least three independent experiments, each with two replicates ± SE.

Interestingly, light grown seedlings showed significantly higher capacity to accumulate proline under NaCl stress when compared to etiolated seedlings both under light and under continuous darkness, irrespective of the concentration of sucrose in the growth medium (Figs. 3*B*, 4*B*). Higher potential of light grown seedlings to accumulate proline in comparison to etiolated seedlings could be due to the presence of well-developed chloroplasts/plastids in the former. Chloroplast is well documented to be the major site for synthesis of proline under stress (Rayapati *et al.* 1989, Sivakumar *et al.* 1998).

The present investigations support the existence of two distinct pathways for proline accumulation in plants under stress; a photo-sensitive and a photo-insensitive. Further, from the results it can also be inferred that sucrose promotes photo-insensitive pathway but suppresses the photosensitive pathway for proline accumulation. Our experiments conclusively prove that light promoted enhancement in stress induced proline accumulation is regulated through processes other than photosynthesis.

References

Alia, Pardha Saradhi, P.: Proline accumulation under heavy metal stress. - J. Plant Physiol. **138**: 554-558, 1991.

Alia, Pardha Saradhi, P.: Suppression in mitochondrial electron transport is the prime cause behind stress induced proline accumulation. - Biochem. Biophys. Res. Commun. 193: 54-58, 1993.

Arora, S., Pardha Saradhi, P.: Light-induced enhancement in proline levels in *Vigna radiata* exposed to environmental stresses. - Aust. J. Plant Physiol. **22**: 383-386, 1995.

Aspinall, D., Paleg, L.G.: Proline accumulation: Physiological aspects. - In: Paleg, L.G., Aspinall, D. (ed.): The Physiology and Biochemistry of Drought Resistance in Plants. Pp. 215-228, Academic Press, Sydney 1981.

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

Dash, M., Panda, S.K.: Salt stress induced changes in growth and enzyme activities in germinating *Phaseolus mungo* seeds. - Biol. Plant. **44**: 587-589, 2001.

Delauney, A.J., Verma, D.P.S.: Proline biosynthesis and osmoregulation in plants. - Plant J. **4**: 215-223, 1993.

Fedina, I.S., Popova, A.V.: Photosynthesis, photorespiration and proline accumulation in water stressed pea leaves. - Photosynthetica **32**: 213-220, 1996.

Greenway, H., Setter, T.L.: Accumulation of proline and sucrose during the first hour after transfer of *Chlorella emersonii* to high NaCl. - Aust. J. Plant Physiol. **6**: 69-79, 1979.

Hong, Z., Lakkineni, K., Zhang, Z., Verma, D.P.: Removal of feedback inhibition of delta(1)-pyrroline-5-carboxylate synthetase results in increased proline accumulation and protection of plants from osmotic stress. - Plant Physiol. **122**: 1129-1136, 2000.

Joyce, P.S., Paleg, L.G., Aspinall, D.: The requirement for low-intensity light in the accumulation of proline as a response to water deficit. - J. exp. Bot. **35**: 209-218, 1984.

Mansour, M.M.F.: Nitrogen containing compounds and adaptation of plants to salinity stress. - Biol. Plant. **43**: 491-500, 2000.

Nikolopoulos, D., Manetas, Y.: Compatible solutes and *in vitro* stability of *Salsola soda* enzymes: proline incompatibility. - Phytochemistry **30**: 411-413, 1991.

Noguchi, M., Koiwai, A., Tamaki, E.: Studies on nitrogen metabolism in tobacco plants. - Agr. biol. Chem. **30**: 452-456, 1966.

Pardha Saradhi, P., Alia, Arora, S., Prasad, K.V.S.K.: Proline accumulates in plants exposed to UV radiation and protects them against UV induced peroxidation. - Biochem. Biophys. Res. Commun. **209**: 1-5, 1995.

Pesci, P.: Glucose mimics the enhancing effect of light on ABA-induced proline accumulation in hydrated barley and wheat leaves. - J. Plant Physiol. **142**: 355-359, 1993.

Rayapati, P.J., Stewart, C.R., Hack, E.: Pyrroline-5-carboxylate reductase is in pea *(Pisum sativum)* leaf chloroplast. - Plant Physiol. **91**: 581-586, 1989.

Sivakumar, P., Sharmila, P., Pardha Saradhi, P.: Proline suppresses Rubisco activity in higher plants. - Biochem. Biophys. Res. Commun. **252**: 428-432, 1998.

Stewart, C.R.: Role of carbohydrates in proline accumulation in wilted barley leaves. - Plant Physiol. **61**: 775-778, 1978.

Stewart, C.J., Morris, C.J. , Thompson, J.F.: Changes in amino acid content of excised leaves during incubation. I. Role of sugar in accumulation of proline in wilted leaves. - Plant Physiol. **41**: 1585-1590, 1966.