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Water Stress-induced Changes in Proline Contents and Nitrate Reductase Activity in Rice Under Light and Dark Conditions

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ABSTRACT

The communication reports water induced changes in proline contents and *in vivo* nitrate reductase (NR) activity in *Oryza sativa* L. Var 'Kranti', under light and dark conditions. Accumulation of free proline was greater in shoot/leaf in polyethylene glycol induced stress, more so, under light conditions as compared to dark. The difference between percentage increase in proline accumulation was less under light/dark conditions, if seedlings were preincubated in 5mM glucose, indicating the role of glucose in light induced proline accumulation. Nitrate reductase activity showed a decline under stress conditions. In light conditions NR activity was greater than in the dark ones in all the treatments including unstressed seedlings.

It is evident from the results that water stress increases free proline content and brings down NR activity in rice, and exposure to light enhances proline content and NR activity both under stress and normal conditions.

In general, the internal water status of plant is a reflection of soil moisture content (Gardner and Ehlig, 1965; Miller et al., 1970). At times, osmotic solutions are employed to impose water stress in plants by exposing the root system to the solutions (Hohl and Peter, 1991; Agarwal et al., 1994). Polyethylene glycol and mannitol are frequently used for this purpose. However, polyethylene glycol appears to be better suited as an external osmoticum to analyze water relations in plants (Hohl and Peter 1991). There are reports which consider stress-induced proline accumulation to be dependent on light intensities and carbohydrate reserve of plants. However, Joyce et al. (1984) indicated that photosynthesis may not be the only factor responsible for increased proline accumulation. Sahoo and Sahu (1993) noticed increased proline accumulation in excised rice leaves in dark as compared in the leaves exposed to light. However, Arora and Pardha Saradhi (1995) recorded increased prolin content in *Vigna radiata* (L.) seedling under condition sof stress and this enhancement was greater in light than in dark. Since, such an enhancement was noticed even in the experiments conducted one roots, they implicated some factor(s) or process(es) other than potosynthesis in light induced enhancement in poline accumulation.

In view of the above referred reports, the present study was undertaken to get a clearer picture of the influence of stress imposed through different osmotica (PEG '6000' and mannitol; that will also give an assessment about the efficacy of each one for the purpose) under light and dark condiions. Proline accumulation and nitrate reductase (NR) activity were monitored, which as such do not appear to possess any direct relationship with each oher but merit individual attention under stress conditions.

MATERIALS AND METHODS

Seeds of rice (*Oryza sativa* L. Var 'Kranti') were procured from Bagwi farm, an extension centre of JNKVV, Jabalpur. Four day old seedlings were incubated in solutions of osmoticum, mannitol 0.3 and 0.6 molal and polyethylene glycol 6000 (027 and 0.3 g/g H_20 approximately equivalent to Ψ=-17 bars and Ψ = -21 bars) for 18 h under light (exposure to two 60 watts Philips spotline NR 63 bulbs through a water jacket) and dark conditions. Water potential of external osmotic was set up using equations i.e., Ψ MAN = -0.079 (MAN) T-22.67 (MAN) for Mannitol and Ψ PEG =1.29 $(PEG)^2$ T-140 $(PEG)^2$-4[PEG] for polyethylene glycol as given by Michel et al. (1983).

In order to see whether preincubation with glucose can mimic the effect of light, in separate set of experiments, both light and dark sets of seedlings were preincubated for 2 h in 5mM glucose before subjecting them to stress and/or light and dark treatments. For field samples, around 30-d after transplantation, the plants were subjected to water stress by with-holding water for one set (till the first leaf unfolded started wilting) and treating the other set as unstressed (where normal submergence was maintained). Third leaf from top was processed as sample.

Subsequently, shoot and root of seedlings were separately processed for free proline contents, method of Bates et al. (1973) was used. *In vino* nitrate reductase (NR EC 1.6.6.1) activity was determined following the method of Srivastava (1974). 0.3 g fresh material was cut into small pieces and put in 5 mL tubes filled with incubation medium [0.1 M phosphate buffer at 7.5 pH and 200 mM KNO_3 and 0.5% n-propanol] and left in dark for two hours at 30º C. Thereafter, to 1 mL aliquot, 1 mL sulfanilamide (1% in 3N HCl) and 1 mL 0.02% naphthyl ethylenediaminehydrochloride was added and shaken thoroughly. After keeping for twenty five minutes for color development absorbance was read at 540 mn. NR activity was calculated employing standard curve of nitrate and expressed in micromole/h/g on a fresh weight basis. For each experiment an average of three to four replications was deduced and standard error computed.

RESULTS AND DISCUSSION

Proline content increased in shoots of stress-inducing seedlings. The increase was more conspicuous in polyethylene glycol-induced stress rather than in mannitol-induced stress. Normally polyethylene glycol is preferred over mannitol as an external osmoticum for experiments on water relations, the reason assigned for this preference is the entry of mannitol into the apoplastic region and symplast (Hohl and Peter, 1991). Our experiments also gave the same indication. In roots proline enhanced slightly that too, in PEG-induced stress. Stress induction by with-holding water to the matured plants (40 d after transplantation) also resulted in increased proline content.

Light-induced enhancement in shoot proline content was noticed under unstressed conditions as well. However, this increase was greater in the seedlings subjected to PEG-induced stress. Promotion of stress-induced proline accumulation by light in higher plants has been recorded by earlier workers (Joyce et al., 1984; Venekamp et al., 1989). On the contrary, Sahoo and Sahu (1993) noticed opposite effects of L-glutamate on senescence induced proline accumulation in dark and light conditions. They are of the opinion that probably senescence was delayed in light and that resulte in senescence induced proline accumulation. Arora and Pardha Saradhi (1995) have however, noticed more proline accumulation in stressed Vigna unguiculata under light conditions that in dark. They have projected the possibility of involvement of photosynthesis only.

In water stressed pea leaves also proline accumulation has been reported with decreasing relative water content, but the increase was greater under light exposed conditions (Fedina and Popova, 1996). Proline synthesis in dark can take place with decreasing leaf water potentials and in the presence of glucose (Fedina and Popova, 1996). In our experiments percentage increase in proline under light conditions over dark is narrowed if the seedlings are preincubated in glucose (5 mM) before stress imposition, particularly under stress conditions. Hence, our results corroborate Fedina and Popova's (1996) observations and the suggestion that availability of energy rich compounds generated from photosynthesis may be the cause of light stimulation of proline synthesis. On the contrary, Greenway and Setter (1979) found that the addition of glucose in dark did not increase proline synthesis.

Stress resulted in reduced NR activity both in roots and in shoots. NR activity was more under light conditions as compared to dark. Light is one of the important factors regulating the inducible nitrate reductase and glucose/sucrose are reported to mimic the light induction of NR m-RNA (Lillo, 1994). However, we did not get consistency in results after preincubation in glucose (results not shown). Table I shows the reduction in NR activity caused by the stress induced by with-holding water to the matured plants (soil moisture contents also given in the same table). Figs. 5 and 6 show lowered values of percentage moisture content in both shoot and root due to incubation of seedlings in different osmoticum solutions of PEG and mannitol suggestive of stress experienced by seedlings under such conditions. Polyethylene glycol induced stress caused in free amino acids and reduction in NR activity in pearl millet (Hanson et al., 1981; Hanson et al., 1982) also. Stress induced decline in nitrate reductase activity has also been noticed by Sarkar et al. (1991). The mode of stress imposition adopted by different workers has been different, that may be the reason for certain variation in the results. Under laboratory conditions stress imposition through polyethylene glycol has given more consistent results than exposure to mannitol, Light-induced enhancement in proline accumulation and NR activity was obvious, and this enhancement in proline was less in the seedlings preincubated with glucose.

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