



SHORT COMMUNICATION

PEG INDUCED MOISTURE STRESS: SCREENING FOR DROUGHT TOLERANCE IN RICE

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A lab experiment was carried out to assess the effect of PEG-6000 induced short term moisture stress on drought tolerance of ten rice genotypes, on the basis of change in some important physico – chemical parameters like proline content, RWC and NR activity in germinating seedlings. Proline contents of seedlings increased with increasing stress; at highest level of imposing stress, i.e. at -10.0 bars of water potential the percentage increment in respect to control represented an order like IET-11120 > CSAR-13 > CSAR-77. Maximum relative water content (RWC) was found in the genotypes CSAR-13 > CSAR-77 > IET-11120 at -10.0 bars of water potential and minimum percentage reduction of nitrate reductase activity in respect to their controls observed in the variety CSAR-77 and followed by IET-11120 > CSAR-27. Hence, the rest of the genotypes were found more sensitive towards increasing stress.

Key words: Nitrate reductase, PEG-6000, proline, rice, RWC.

Water stress affects cell water potential, movement of stomata, rate of photosynthesis, nitrate assimilation and a number of anabolic reactions (Sairam *et al.* 1990). Plants exposed to various environmental stresses generate/activate a number of defense mechanism/metabolic changes for their survival. Genes corresponding to various stresses and their resultant products were analyzed in Arabidopsis and in rice (Seki *et al.* 2002, Rabbani *et al.* 2003). Moisture stress induced rice cultivars showed higher proline and lower NR activity in their growing shoot (Manabendra *et al.*, 1998). The proline accumulation under drought condition is a close indicator of drought resistance/tolerance capacity of plant. Singh and Singh (1983) observed that the proline accumulation increased with increasing stress level. PEG-6000 appears to be better suited as an external osmoticum to analyze water relation in plants (Hohl and Peter 1991). In view of the above referred reports, the present study was undertaken to get a clear picture of

the influence of moisture stress, imposed through different osmotica of PEG-6000 to screen the drought tolerant genotypes of rice.

Short-term petridish experiment was conducted with ten rice cultivars procured from the research farm of C.S. Azad University of Agriculture and Technology, Kanpur (U.P.). Various concentrations of PEG-6000 were made to get -5.0 and -10.0 bars of water potential by using the method of Hadas (1976). Distilled water was used for control. Twenty five surface sterilized seeds were placed in each Petri dish on the filter paper, moistened with PEG-6000 solutions to maintain the stress level. The petridishes were kept under normal light at room temperature. RWC of the seedlings were calculated as per the method of Barrs and Weatherly (1962).

Spectrophotometric (Model Digispec-110D of Sico) analysis of proline content and nitrate reductase activity

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of the shoot portion of the rice seedlings were carried out by using the methods of Bates *et al.* (1973) and Klepper *et al.* (1971) respectively. All the experiments were repeated thrice. The experiments followed the completely randomized design and statistical analyses were done as per requirement.

Proline accumulation was studied in 192 h germinated rice seedlings in stressed, (imposed by using

PEG-6000) and non stressed conditions (Table 1) Proline content sharply increased with increasing stress from -5.0 to -10.0 bars of water potential in each of the ten tested varieties. The maximum increment found in IET-11120, i.e. about 615% at -10.0 bars of water potential treatment in respect to its non stressed control seedlings. The 2nd and 3rd positions in respect to an increment in proline accumulation were achieved by CSAR-13 and CSAR-77 at highest range of induced moisture stress.

Table 1. Effect of external water potential treatments, maintained by PEG-6000, on proline content ($\mu\text{g g}^{-1}$ fw), RWC (%) and nitrate reductase activity (nmol g^{-1} fw h^{-1}) in rice seedlings at 192 h.

Cultivars /Treatment	Proline content			Relative water content			Nitrate reductase activity		
	Treatment			Treatment			Treatment		
	Cont.	-5.0 bar	-10.0 bar	Cont	-5.0 bar	-10.0 bar	Cont.	-5.0 bar	-10.0 bar
CSAR-13	30.1 (0.0)*	69.8 (+131.9)*	142.8 (+374.4)*	82.0 (0.0)*	72.0 (-12.20)*	55.0 (-32.93)*	1700 (0.0)*	1400 (-17.65)*	1200 (-29.4)*
CSAR-27	26.4 (0.0)*	46.2 (+75.0)*	87.6 (+231.8)*	85.0 (0.0)*	64.0 (-24.71)*	46.0 (-45.88)*	1500 (0.0)*	1200 (-20.0)*	1100 (-26.7)*
CSAR-77	31.9 (0.0)*	72.1 (+126.0)*	140.2 (+339.5)*	84.0 (0.0)*	69.0 (-17.9)*	53.0 (-36.90)*	1600 (0.0)*	1500 (-6.25)*	1300 (-18.8)*
CSAR-148-205	22.7 (0.0)*	45.8 (+101.8)*	81.2 (+257.7)*	85.0 (0.0)*	65.0 (-23.53)*	48.0 (-43.53)*	1600 (0.0)*	1300 (-18.8)*	1100 (-31.3)*
CSAR-253	19.9 (0.0)*	55.0 (+176.3)*	85.6 (+330.2)*	86.0 (0.0)*	62.0 (-27.91)*	46.0 (-46.52)*	1700 (0.0)*	1500 (-11.8)*	1200 (-26.41)*
CSAR-256	25.0 (0.0)*	69.2 (+176.8)*	102.3 (+309.2)*	90.0 (0.0)*	67.0 (-25.56)*	40.0 (-55.56)*	1500 (0.0)*	1300 (-13.3)*	1000 (-33.3)*
Pant-12	22.6 (0.0)*	46.4 (+105.31)*	82.1 (+263.3)*	84.0 (0.0)*	69.0 (-17.86)*	44.0 (-47.62)*	1600 (0.0)*	1400 (-12.50)*	1100 (-31.3)*
Basmati-370	18.3 (0.0)*	44.8 (+144.8)*	78.6 (+329.5)*	86.0 (0.0)*	65.0 (-24.53)*	46.0 (-46.51)*	1500 (0.0)*	1200 (-20.0)*	1100 (-26.7)*
IET-11120	21.9 (0.0)*	62.8 (+168.8)*	156.6 (+615.0)*	83.0 (0.0)*	68.0 (-18.10)*	52.0 (-37.53)*	1700 (0.0)*	1400 (-17.7)*	1300 (-23.53)*
IR-539-30-2-2-3-3	20.7 (0.0)*	42.8 (+106.8)*	78.2 (+277.9)*	84.0 (0.0)*	69.0 (-17.86)*	44.0 (-47.62)*	1600 (0.0)*	1200 (-25.0)*	1100 (-31.3)*
Variety	SE \pm (diff) 1.390 CD (at 5%) 2.758			SE \pm (diff) 0.883 CD (at5%) 1.355			SE \pm (diff) 0.035 CD (at 5%) 8.900		
Treatment	SE \pm (diff) 0.883 CD (at 5%) 1.950			SE \pm (diff) 0.463 CD (at5%) 0.958			SE \pm (diff) 0.024 CD (at 5%) 0.049		
V \times T	SE \pm (diff) 3.010 CD (at 5%) 6.188			SE \pm (diff) 1.527 CD (at 5%) 3.030			SE \pm (diff) 0.078 CD (at 5%) 0.015		

*Per cent increase / decrease with respect to control

However, CSAR-13 and CSAR-77 had also more proline content even in non stressed one in comparison to other tested genotypes (Table 1). The relative water content in every tested cultivars of rice at 192 h of germination were found to reduce with increasing stress from -5.0 to -10.0 bars of water potential as compared to their non stressed partners but the least per cent decrease (given in parenthesis) was found in CSAR-13 followed by CSAR-77 and IET-11120 (Table 1).

Stress resulted in a significant reduction in nitrate reductase activity in all the tested genotypes as compared to their non stressed control. However, least reduction in nitrate reductase activities at highest level of stress, i.e. -10.0 bars of water potential was noticed in CSAR-77 followed by IET-11120 and CSAR-27 as compared to their non stressed control. An increased activity of enzyme protease accompanied by increased free proline was observed in germinating rice seedlings, imposed with short term (18 h) stress (Pandey *et al.* 2004). Chandrasekar *et al.* (2000) reported that water stress caused a decline in relative water content (RWC), chlorophyll and carotenoid contents, membrane stability, nitrate reductase activity and increased proline accumulation in all tested wheat genotypes. Polyethylene glycol induced stress caused a reduction in nitrate reductase activity in pearl millet and in soybean (Hanson *et al.* 1981, Hanson *et al.* 1982, Sarkar *et al.* 1991). Present study also supports these findings. Thus the over all result suggested that CSAR-13, IET-11120 and CSAR-77 are more resistant towards the higher range of moisture stress among tested genotypes of rice.

REFERENCES

- Barrs, H.D. and Weatherley, P.E. (1962). A re-examination of the relative turgidity techniques for estimating water deficits in leaves. *Aust. J. Biol. Sci.* **15**: 413-428.
- Bates, L.S., Waldren, R.P. and Teare, I.D. (1973). Rapid determination of free proline for water stress studies. *Plant & Soil* **39**: 205-207.
- Chandrasekhar, V., Sairam, R.K. and Srivastava, G.C. (2000). Physiological and biochemical responses of hexaploid and tetraploid wheat to drought stress. *J. Agron. Crop Sci.* **185**: 219-227.
- Hadas, A. (1976). Water uptake and germination of leguminous seed under changing external water potential in osmotic solution. *J. Exp. Bot.* **27**: 480-489.
- Hanson, I.E., Mahalakshmi, V., Biolenger, F.R. and Alagarwamy, G.A. (1981). Stomatal response of pearl millet (*Pennisetum americanum* L.) genotypes in relation to abscisic acid and water stress. *J. Exp. Bot.* **32**: 1211-1221.
- Hanson, I.E., Alagarwamy, G.A., Mahalakshmi, V. and Biolenger, F.R. (1982). Diurnal changes of endogenous abscisic acid in leaf of pearl millet (*Pennisetum americanum* L.) under field condition. *J. Exp. Bot.* **33**: 416-425.
- Hohl, M. and Peter (1991). Water relation of growing maize coleoptiles. Comparison between mannitol and polyethylene glycol-6000 as external osmotica for adjusting turgor pressure. *Plant Physiol.* **95**: 716-722.
- Kleeper, L., Flesher, D. and Hageman, R.H. (1971). Generation of reduced nicotinamide dinucleotide for nitrate reduction in green leaves. *Plant Physiol.* **48**: 580-590.
- Manabendra, D., Baruah, K.K. and Deka, M. (1998). Moisture stress induced changes in seed germination and seedling growth of upland 'Ahu' rice (*Oryza sativa* L.). *Indian J. Ecol.* **25**: 133-137.
- Pandey, Rashmi, Agrawal, R.M., Jeevaratnam, K. and Sharma, G.L. (2004). Osmotic stress – induced alterations in rice (*Oryza sativa* L.) and recovery of stress release. *Plant Growth Regul.* **42**: 79-87.
- Rabbani, M.A., Maruyama, K., Abe, H., Khan, M.A., Katsura, K., Ito, Y., Yoshiwara, K., Seki, M., Shinozaki, K. and Yamaguchi-Shinozaki, K. (2003). Monitoring expression profiles of rice genes under cold, drought and high-salinity stresses and abscisic acid application using cDNA microarray and RNA gel-blot analyses. *Plant Physiol.* **133**: 1755-1767.
- Sairam, R.K., Desmukh, P.S., Shukla, D.S. and Ram, S. (1990). Metabolic activity under moisture stress in wheat genotypes. *Indian J. Plant Physiol.* **33**: 226-231.
- Sarkar, R.K., Saini, J.P. and Dubey, C.D. (1991). Testing of soybean (*Glycine max* L.) genotypes for drought tolerance. *J. Agric. Sci.* **61**: 369-373.
- Seki, M., Narusaka, M., Ishida, J., Nanjo, T., Fujita, M., Oono, Y., Kamiya, A., Nakajima, M., Enju, A. and Sakurai, T. (2002). Monitoring the expression profiles of 7000 *Arabidopsis* genes under drought, cold, and high-salinity stresses using a full-length cDNA microarray. *The Plant J.* **31**: 279-292.
- Singh, K.P. and Singh, K. (1983). Influence of stimulated water stress on free proline accumulation in *Triticum aestivum* L. *Indian J. Plant Physiol.* **26**: 319-321.