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Drought Induced Changes in Proteins and Superoxide Dismutase Zymogram of Rice Cultivars

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ABSTRACT. Seventeen rice genotypes were screened for seminal root elongation under rapid screening test. Three top ranking genotypes along with one susceptible check, were again tested for germination and seedling growth in D-mannitol induced water stress. Western analysis was done with the antibodies of heat shock protein (HSP) 90 and HSP 104 to find the proteins specifically expressed in water stressed roots and seed calli. The changes in peroxidase and superoxide dismutase (SOD) activities were studied in roots under water stress. Change in superoxide dismutase activity was further analysed using zymogram of water stressed roots and seed calli. Wide genotypic variation was noticed for seminal root elongation and germination, and seedling growth under water stress among the genotypes.

INTRODUCTION

Drought is a major constraint limiting rainfed rice production both in upland and lowland ecosystems. Deep and extensive root system that fully extracts available soil water has the potential to increase yield under drought. Thickness, length and soil hardpan penetration ability of roots are associated with drought tolerance in rice (Ingram *et al.*, 1994). Incorporation of root traits into breeding program has been difficult due to the labourious nature of measuring root characters (O'Toole, 1989). While root-specific genes of unknown function have been cloned from rice, genes governing root-growth and drought adaptation have not been isolated (Toenniessen, 1991). Identifying genes regulating root traits and root response to water stress could be used to develop rice cultivars better-adapted to water deficit environments.

Changes induced by drought are generally accompanied by increases or decreases in a relatively small set of cellular proteins. Some of the water stress induced proteins, mostly from shoot tissues, have been purified and characterized. The production of both low and high molecular weight heat shock proteins (HSPs), has been related to tolerance to many abiotic stresses in several crop plants including rice (Grover *et al.*, 1998). HSPs are considered to play an important role in repairing or protecting against damage to cellular components. They are further implicated to function as molecular chaperones as well as in the degradation of stress damaged proteins which may other wise be harmful to cellular functions.

Genotypic variation in drought resistance is known in rice and certain cultivars are relatively better adapted to drought (Ingram *et al.*, 1994). The biochemical changes taking place in drought sensitive and tolerant genotypes under stress has been poorly

studied in rice, more so in roots. Since root system plays a major role in drought resistance in rice, understanding the biochemical changes taking place in roots under stress will help in identifying the genes linked to drought tolerance. Thus the study was made to find out water stress induced changes in certain proteins and enzymes in the roots and callus of drought tolerant and sensitive rice genotypes.

MATERIALS AND METHODS

Seminal root elongation

Seventeen rice genotypes were initially tested for their relative drought tolerance under rapid screening test. This screening test has been standardised to rank rice germplasm for drought tolerance (Gomathinayagam and Natarajan, 1988). Briefly, pregerminated seeds were placed over glass beakers filled with distilled water and covered using a nylon net. The water in the beaker was drained every day so that only 3 mm length of the longest seminal root remained in contact with water. The length of the longest root was measured 10 days after sowing (DAS).

D-mannitol test

The genotypes *viz.*, Nootripathu, Kallurundaikar, Norungan and IR 20 were chosen for further tests based on the rapid screening test. The former 3 genotypes had longer roots, while IR 20 had shorter root. Seeds of these lines were germinated in Whatman No. 1 filter paper circles mounted on petriplates using D-mannitol solutions of 1, 5 and 10% concentrations. Twenty seeds per plate with 4 plates per genotype per treatment were maintained. The germination, root and coleoptile lengths were recorded 10 DAS.

Collection of water stressed roots

The genotypes *viz.*, Noortipathu, Norungan, Kallurundaikar and IR 20 were raised in mud pots of 30×35×30 cm dimension filled with potting mixture comprising soil:sand:farm yard manure in 1:1:1. Two plants per pot were maintained. The plants were fully irrigated up to 60 DAS and irrigation was withheld for the stressed plants. Relative water content (RWC) of the 2nd youngest fully expanded leaf was monitored regularly (Barrs and Weatherley, 1962). Young root tissues without browning were sampled from the stressed plants when their leaf RWC declined to 70–75%. Simultaneously root samples were also collected from non-stressed control plants.

In vitro stress treatment for seed callus

The seed callus of the selected genotypes was raised in MS medium (Murashige and Skoog, 1962) and the calli were subjected to stress *in vitro* by subculturing them in modified MS medium + 1 mg 2, 4 dichlorophenoxyacetic acid + 0.75 mg l^{-1} kinetin + 1%

D-mannitol. Fifteen day old subcultured seed calli from normal and mannitol added MS media were analysed for changes in proteins.

Western blotting

The fresh roots and the subcultured callus samples were ground using 10 mM phosphate buffer (pH 7.2) containing β-mercaptoethanol and 2 mM phenyl methyl sulfonyl fluoride. The supernatant was collected and the proteins were separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (Laemmli, 1970). The gel was then blotted on to a nitrocellulose membrane. The HSP 90 and HSP 104 proteins were immunodetected by the antibodies at dilutions of 1:25,000 and 1:50,000 respectively.

Peroxidase and SOD activities

Fresh root samples were homogenised using 50 mM phosphate buffer (pH 7.0) and the extracts were used for enzyme estimation. The reaction mixture consisted of 0.1 ml of enzyme extract, 3 ml of phosphate buffer, 0.05 ml of guaiacol and 0.03 ml hydrogen peroxide. Peroxidase activity was determined by recording the time taken for the increase in absorbance by 0.1 from the initial absorbance of 0.05 at a wavelength of 436 nm and expressed in units l^{-1}. For SOD assay, 3 ml of reaction mixture consisting of 50 mM potassium phosphate buffer (pH 7.8), 13 mM methionine, 2 mM riboflavin, 0.1 mM ethylene diamine tetra acetic acid (EDTA) and 75 mM nitroblue tetrazolium (NBT) was taken along with 5 μl enzyme extract and absorbance read at 560 nm. The tubes were exposed to fluorescent light for 2–15 min before the absorbance was read. One unit of SOD activity was expressed as 50% reduction of absorbance (Beauchamp and Fridorich, 1971).

SOD zymogram

Polyacrylamide gel electrophoresis was carried out in the absence of SDS and the gels were run at a temperature of 10–12°C. The gel was immediately transferred to the staining solution containing 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 3.0 mM riboflavin, 0.25 mM NBT and 0.2% tetraethyl methylene ethylene diamide for 20 min. The gel was washed with distilled water and exposed to 400 W fluorescent bulbs at a distance of 30–40 cm for 5 min at room temperature.

RESULTS AND DISCUSSION

Significant variation in seminal root length under rapid screening was noticed among the genotypes. The landrace Nootripathu had the longest roots (Table 1) followed by Norungan and Kallurundaikar. IR 20 had the shortest seminal root. The longer roots may help to access the soil moisture at depth during water stress. The selected genotypes showed differential response to water stress using D-mannitol (Table 2). IR 20 showed reduction in germination as compared to other genotypes even at 1% D- mannitol. IR 20

showed drastic reduction in germination in 5% D-mannitol whereas, Kallurundaikar (67.5%) and Nootripathu (42.5%) recorded considerably higher germination. Under 10% D-mannitol, the germination was very much affected in all genotypes. In 5% mannitol, Kallurundaikar maintained its root length as that in control while the other genotypes showed 4–10 times reduction in root growth. However, the root growth was almost negligible in IR 20. A similar trend was observed in case of shoot height also.

Table 1. Selection of drought tolerant rice cultivars by the rapid screening technique based on the root length parameter under water depletion.

Cultivars	Root length* on 10th day after sowing (cm)	Rank
Basumathi	4.65	8
Chandikar	9.22	4
CO 43	4.65	8
CO 45	6.70	6
IR 20	6.37	7
IR 50	5.07	8
JJ 92	4.70	8
Kallurundaikar	11.10	3
Karunkuruvai	7.05	5
Mattaikar	11.05	3
Nootripathu	14.40	1
Norungan	12.75	2
PM 9106	6.15	7
PMK 1	9.25	4
PMK 2	7.32	5
Ponni	3.50	8
Poongar	7.20	5
CD at 5% P	0.456**	

* Mean of 4 experiments

Using a highly specific polyclonal antibody, the HSP 104 synthesised by water stressed Nootripathu and Norungan root samples as well as their mannitol treated seed calli were detected. It was seen that HSPs are induced in response to water stress in rice (Plate 1-A). This was in agreement with the findings of Almoguera *et al.* (1993).

Table 2. Effect of varying D-mannitol concentrations (1–10%) on germination (%), root and shoot growth (cm) in the 5 selected rice varieties, 10 days after sowing.

Cultivar	Treatment	Germination %	Root length (cm)	Shoot length (cm)	Vigour index
IR 20	Control	95.0	2.5	7.30	238
	1%	87.5	1.7	4.20	149
	5%	7.5	0.2	0.05	2
	10%	0.0	0.0	0.00	0
Karunkuravi	Control	90.0	7.0	4.10	630
	1%	55.0	3.7	2.95	204
	5%	7.5	0.7	0.50	6
	10%	12.5	0.1	0.00	2
Kallurandaikar	Control	100.0	7.6	3.20	760
	1%	100.0	6.5	2.85	650
	5%	67.5	2.8	1.75	192
	10%	22.5	1.1	0.30	25
Nootripathu	Control	100.0	8.5	3.80	850
	1%	95.0	3.0	1.00	285
	5%	42.5	1.2	0.50	53
	10%	15.0	0.6	0.20	10
Norungan	Control	90.0	4.0	2.10	360
	1%	92.5	4.1	2.15	384
	5%	12.5	1.0	0.25	13
	10%	2.5	0.1	0.05	1
CD at 5%		2.0	0.9	0.45	

The water stressed roots and mannitol treated seed callus of the susceptible cultivar IR 20 did not show any reaction to HSP 104. This indicates that HSP 104 may play a crucial role in drought tolerance in rice. The 2 polypeptides of molecular weight 87 and 85 kDa are together referred to as HSP 90. The 87 and 85 kDa polypeptides reacted to form 2 distinct bands indicating that they are expressed during water stress in roots of drought tolerant Nootripathu and Norungan, while the root samples of IR 20 did not show any reaction (Plate 1-B). No bands were observed from the calli subjected to stress, indicating that the magnitude of stress (1% D-mannitol) probably was not adequate to elicit the response.

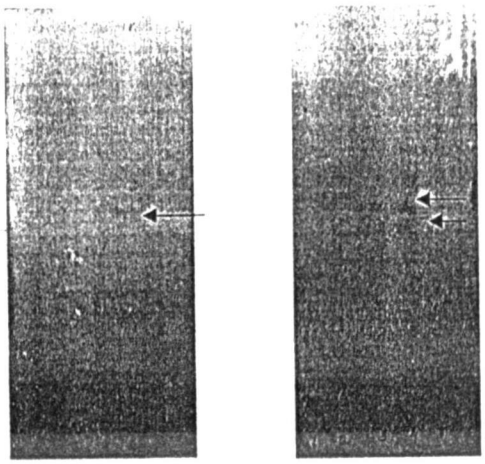

Plate 1. Western blot showing immunodetection of HSP 104 (A) and 90 (B) in root samples of water stressed IR 20 (Lane 2), Nootripathu (L 3), Norungan (L 4) and in callus samples of D-mannitol stressed Nootripathu (L 5) and Norungan (L 6) along with the untreated IR 20 root sample (L 1).

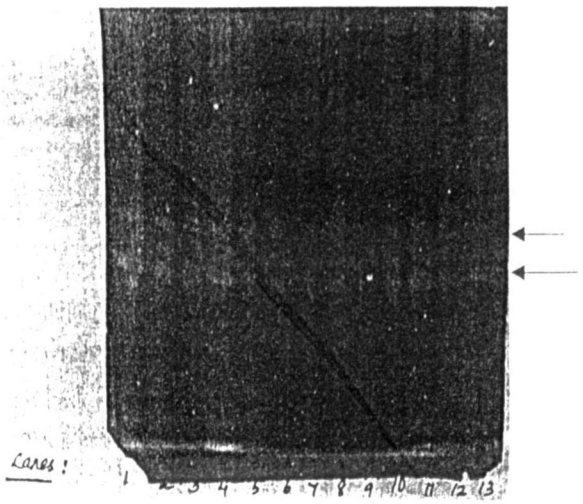

Plate 2. Superoxide dismutase isozyme pattern of the root samples of water stressed IR 20 (Lane 2), Norungan (L 4), Nootripathu (L 10), Kallurundaikar (L 12,13) and callus samples of D-mannitol stressed IR 20 (L 6), Nootripathu (L 8) along with the untreated root samples of IR 20 (L 1), Norungan (L 4), Nootripathu (L 9), Kallurundaikar (L 11) and callus samples of IR 20 (L 5), Nootripathu (L 7).

There was an increase in peroxidase activity during stress in all the genotypes (Fig. 1). The increase was more pronounced in tolerant genotypes *viz.*, Norungan and Nootripathu compared to susceptible IR 20. Similar trend was noticed in SOD activity (Fig. 2). The increased tolerance of some cultivars as compared to others is considered

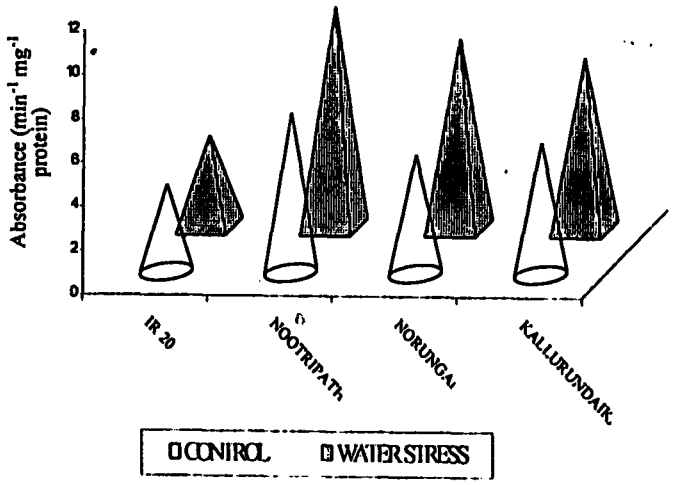

Fig. 1. Effect of water stress on peroxidase in roots of rice cultivars.

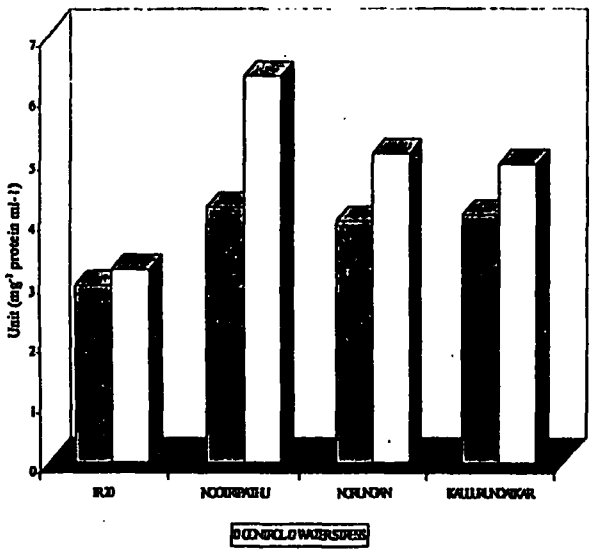

Fig. 2. Effect of water stress on superoxide dismutase in rice cultivars.

to involve the expression of new isoforms of certain enzymes. The isozyme pattern of SOD for the root and callus samples of tolerant and susceptible cultivars were shown (Plate 2). In case of stressed Nootripathu root sample, 2 very distinct isoforms were observed which are otherwise absent in IR 20. This might be a significant finding as these isoforms shall be responsible for the drought tolerance nature of Nootripathu. However, Norungan did not show any distinct bands for SOD indicating that the mechanism of stress tolerance may be genotype specific.

CONCLUSIONS

Water stressed roots of drought tolerant Nootripathu and Norungan showed positive reaction for HSP 90 and 104, while the susceptible IR 20 did not. Peroxidase activity increased in roots under water stress, but the increase was well pronounced in tolerant genotypes. SOD activity also increased in roots under stress, but the increase was several folds higher in drought tolerant, Nootripathu. This rise corresponded to the observation of 2 new isoforms in the SOD zymogram of this genotype in water stressed roots.

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