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EFFECT OF DROUGHT ON PROTEINS AND ISOENZYMES IN RICE DURING GERMINATION*

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Key Word Index—*Oryza sativa*; Gramineae; rice; soluble proteins; isoenzymes; effect of water stress; germination.

Abstract— Two drought tolerant varieties TKM-1 and TKM-2 and two drought susceptible varieties Jaya and Improved Sabarmati of rice were studied for soluble protein pattern and isoenzymes of malate dehydrogenase, glutamate dehydrogenase, esterase and peroxidase during germination at different water stress. MDH, GDH and esterase patterns were not affected, but the soluble proteins were changed. Peroxidase isoenzyme pattern from drought tolerant and susceptible varieties showed characteristic differences. The intensity of bands with higher electrophoretic mobility decreased in Jaya and Improved Sabarmati, while in TKM-1 and TKM-2 the intensity of these bands did not change much after 72 hr water stress. In shoots of Jaya and Improved Sabarmati, the activity of the peroxidase isoenzymes decreased more than in TKM-1 and TKM-2 shoots with increase in water stress.

INTRODUCTION

Plant responses to water stress include changes in enzyme activity, amino acid metabolism and protein synthesis [1]. It has been suggested that a more favourable balance between synthesis and breakdown is maintained by drought resistant variety during water stress than drought susceptible variety [2]. Although activity of several enzymes is influenced by water stress it is not known if isoenzyme patterns are changed. Therefore, in the present study the effect of water stress on soluble protein, peroxidase, MDH, GDH and esterase isoenzymes have been studied in varieties differing in tolerance to water stress.

RESULTS AND DISCUSSION

Soluble protein

The soluble protein pattern obtained on isoelectric focusing from 8-day-old shoots and roots of TKM-1 and Improved Sabarmati (IS) under different moisture stress created by mannitol and polyethylene glycol (PEG) are shown in Fig. 1. The pH gradient obtained ranged from 3.8 to 9. The patterns from TKM-2 were similar to TKM-1 and from Jaya to IS. Most of the bands in shoots and roots were detected between pI 3.9 to 6. A total of 19 bands were seen in the control. In general the soluble protein patterns from TKM-1 and IS were similar when no water stress was applied. The soluble protein pattern from TKM-1 and IS showed both qualitative and quantitative differences when moisture stress was given by PEG. The decrease in intensity of bands with higher pI value (>5) was greater in IS compared to TKM-1. Water stress affected the soluble protein pattern from roots of TKM-1 and IS considerably. The intensity of most of the bands decreased considerably at -7.5 bars osmotic potential (ψs) when mannitol was used.

The soluble protein content in shoots increased with increase in water stress either by PEG or mannitol,

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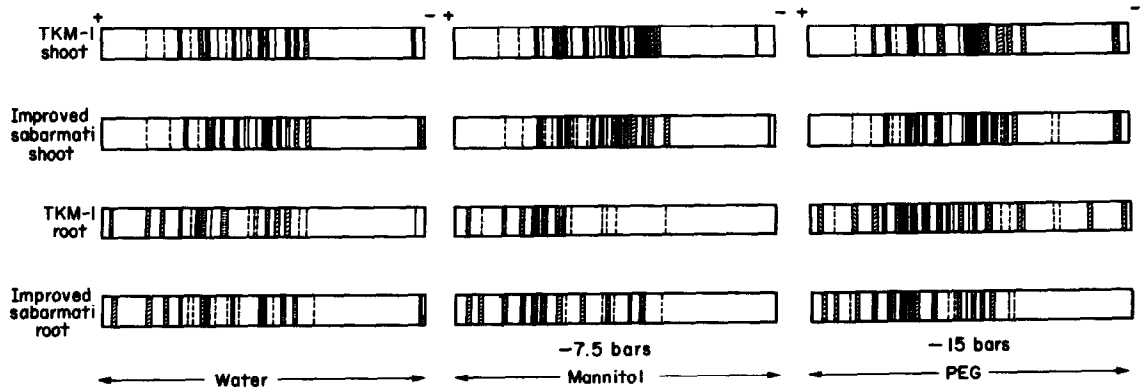

Fig. 1. Soluble protein on isoelectric focusing from TKM-1 and Improved Sabarmati shoot and root (pH gradient left to right 3.9 to 8.4).

Table 1. Effect of moisture stress on soluble protein (mg/g fr. wt) in 8-day-old shoot and root of TKM-1 and Improved Sabarmati

Treatments	TKM-1		Improved Sabarmati	
	Shoot	Root	Shoot	Root
Water	19·8	2·6	19·5	2·0
−5 bars (PEG)	25·4	2·5	19·7	1·6
−10 bars (PEG)	32·8	2·3	22·0	1·5
−15 bars (PEG)	31·9	2·1	26·2	1·4
−2.5 bars (Mannitol)	26·0	1·8	25·3	1·2
−7.5 bars (Mannitol)	28·6	1·7	26·2	1·1

while in roots it decreased (Table 1). The decrease in soluble protein was more in IS roots when compared to TKM-1. The increase in soluble protein in TKM-1 shoots was 65% while in IS it was only 13% at −10 bars ψs by PEG. These results are in agreement with the effect of water stress observed by other workers [3, 4]. The differential effects of mannitol and PEG on soluble protein pattern and amount could be due to the effect of these compounds as it is known that both mannitol and PEG enter the roots. The amount of mannitol entering the root is higher compared to PEG-6000. Stutte and Todd [4] observed changes both in the number and R_m value of protein bands in wheat during water stress. Wheat varieties resistant to water stress have been shown to maintain a higher percentage of high MW proteins under water stress than the susceptible varieties. In the present study also greater changes have been observed in IS compared to TKM-1.

MDH, GDH, and esterase isoenzymes

MDH, GDH and esterase isoenzyme patterns in TKM-1, TKM-2, Jaya and IS were not influenced by moisture stress. Three MDH isoenzymes with R_m 0.44, 0.54 and 0.63 were present in shoots of all the varieties. Two GDH bands at R_m 0.18 and 0.25 were present in Jaya shoots while shoots from other varieties had one band at R_m 0.18. The esterase pattern also did not show any major difference.

Peroxidase

Peroxidase patterns from 72 hr imbibed seeds of TKM-1, TKM-2, Jaya and IS at different water stress are shown in Fig. 2. Peroxidase patterns in TKM-1 and TKM-2 were similar except for a minor band at R_m 0.40 in the former. Jaya showed two additional bands at R_m 0.19 and 0.77 as compared to IS. Bands with R_m 0.08 0.13 and 0.54 were similar in all the varieties. With increase in water stress the intensities of the bands with medium and high electrophoretic mobility decreased and finally at −7.5 bars ψs bands with R_m 0.19, 0.27, 0.54, 0.77 and 0.83 disappeared in Jaya and IS whereas in drought tolerant varieties TKM-1 and TKM-2 the isoenzyme pattern was not affected much at −2.5 and −5.0 bars ψs. Even at −7.5 bars ψs all the bands were still present in TKM-1 and TKM-2 although the intensity of a few bands was reduced.

The peroxidase isoenzyme patterns in shoots of 8, 10 and 12-day-old seedlings of IS and TKM-1 subjected to different water stresses are shown in Fig. 3. The patterns in Jaya were similar to IS except for the difference in the intensity of the bands with R_m 0.58 and 0.88. The pattern in TKM-2 was similar to TKM-1. Peroxidase patterns from TKM-1 and IS showed differences. The decrease in the intensity of peroxidase bands was greater in IS compared to TKM-1 with increase in moisture stress. The effect of water stress on isoenzyme patterns at 10 and 12 day's growth was similar to that observed at the 8 day stage.

The peroxidase isoenzyme patterns in roots at 8, 10 and 12 days' growth are shown in Fig. 4 for IS and TKM-1 respectively. The patterns in Jaya were similar to IS and those of TKM-2 were similar to TKM-1. TKM-1 had 9 peroxidase bands at the 8 and 10 days stage and 10 bands at 12 days stage. IS had 10 bands at all the stages.

The peroxidase isoenzyme pattern was not affected in TKM-1 and TKM-2 by water stress. The intensities of the bands with high and medium electrophoretic mobility increased at −2.5 bars ψs. Even at higher ψs all the peroxidase bands were present, although there was a slight decrease in the intensity at −7.5 bars ψs. In contrast to this the intensity of bands with R_m 0.20, 0.25, 0.54 and 0.89 decreased in Jaya and IS.

MDH, GDH and esterase isoenzymes patterns in rice varieties differing in drought tolerance were not influenced by water stress. This suggests that these enzymes do not play an important role in drought tolerance. Changes observed in soluble protein pattern in the present study are similar to the changes in wheat leaf peroxidase with wilting [4]. The differences observed in peroxidase isoenzymes between the drought tolerant varieties (TKM-1, TKM-2) and drought susceptible varieties

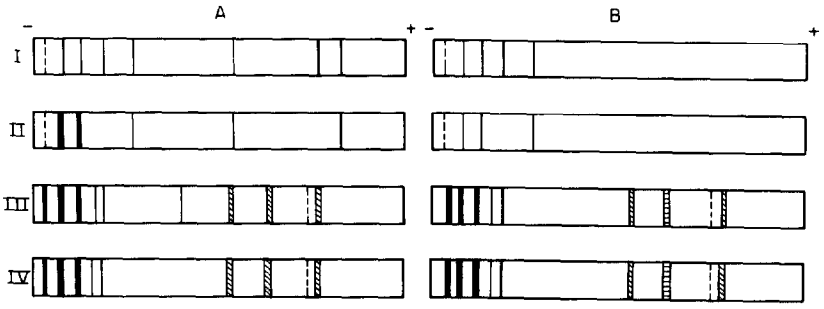

Fig. 2. Peroxidase isoenzyme pattern after 72 hr germination under water stress, I Jaya, II—Improved Sabarmati, III—TKM-1, IV—TKM-2, (A) Water, (B) −7.5 bars osmotic potential with mannitol.

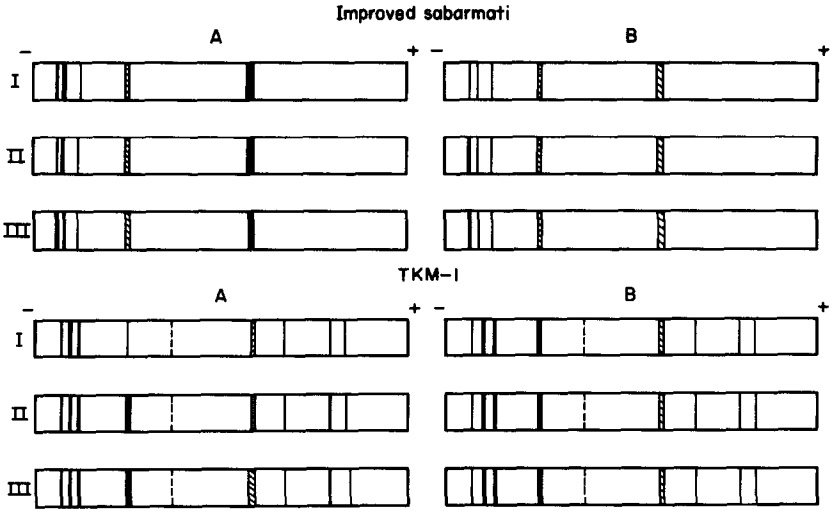

Fig. 3. Peroxidase isoenzyme pattern in shoots of TKM-1 and Improved Sabarmati. Shoot: I—8 days, II—10 days, III—12 days, (A) Water, (B) −7.5 bars with mannitol.

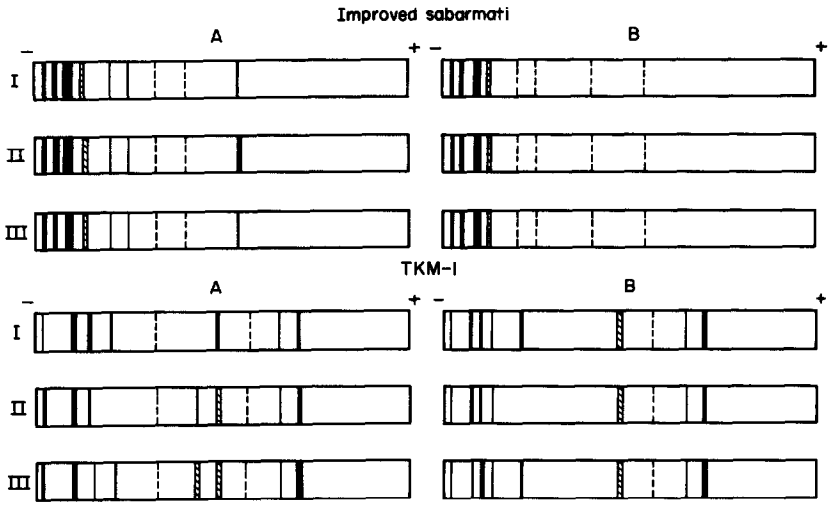

Fig. 4. Peroxidase isoenzyme pattern in TKM-1 and Improved Sabarmati roots during water stress. Root: I—8 day, II—10 day, III—12 day, (A) Water, (B) −7.5 bars with mannitol.

(Jaya, IS) are of interest, as peroxidase isoenzymes are less affected by moisture stress in the former. The exact physiological role of peroxidase in plants remains obscure. Interaction of growth hormones such as IAA with peroxidase might play an important role in growth regulation [5]. Therefore the differential effects observed during water stress between drought tolerant and susceptible rice varieties are perhaps due to their adaptibility to drought.

EXPERIMENTAL

Seeds sterilized by treatment with 0.1% $HgCl_2$ for 1 min were washed with H_2O and allowed to germinate at 28° in Petri dishes lined with filter paper discs wetted with H_2O or with solns of different concs of mannitol. For studies relating to 5 day's growth or more, the seeds were planted in wet vermiculite in an illuminated germinating chamber at 28°. The stress was created by transferring seedlings to solns of different concs of mannitol or PEG-6000 in covered test tubes and kept in the illuminated germinating chamber at 28° for 2 days. The conc of PEG-6000 for different osmotic potential was according to ref. [6].

Soluble proteins. Germinated seeds, shoots or roots were extracted by hand grinding in a chilled pestle and mortar with 50 mM Tris–Cl buffer (pH 7.6) containing 5 mM 2-mercaptoethanol and 5 mM EDTA for soluble protein and isoenzymes of MDH, GDH and esterase. Mercaptoethanol and EDTA was omitted for peroxidase. All the operations were carried out at 4°. The cell paste suspension was centrifuged at 10000 *g* for 20 min at 0° and the supernatant used for isoenzyme studies. Isoelectric focusing of soluble protein and staining was carried out as described in ref. [7]. Mobility (R_m) is relative to the mobility of the tracking dye. The anionic system of polyacrylamide gel electrophoresis was used to separate various isoenzymes. MDH bands were developed according to the method used in ref. [9]. GDH and esterase bands were developed after electrophoresis according to ref. [8]. Densitometer tracings of gels were obtained on a Joyce-Loebl Chromoscan.

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