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EFFECT OF DROUGHT ON ENZYMES AND FREE PROLINE IN RICE VARIETIES*

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Key Word Index—*Oryza sativa*; Gramineae; rice; water stress; peroxidase; RNase; protease; nitrate reductase; free proline; germination.

Abstract—Drought tolerant rice variety TKM-1 and susceptible variety Improved Sabarmati (I.S.) showed characteristic differences in peroxidase, RNase, nitrate reductase, protease and in free proline accumulation during water stress. Water stress resulted in greater decrease in peroxidase activity in I.S. compared to TKM-1 in 72 hr imbibed seeds. RNase decreased in TKM-1 roots and increased in I.S. roots with increase in water stress. Protease activity was found to be higher in TKM-1 compared to I.S. Protease activities in TKM-1 shoots increased with water stress while in I.S. shoots it did not change much. In TKM-1, a 5.4 fold increase in free proline occurred as compared with a 1.2 fold increase in I.S. at −10 bars osmotic potential.

INTRODUCTION

Plants differ markedly in their tolerance to drought. Several aspects of metabolism such as inhibition of protein synthesis and changes in amino acid metabolism have been shown to be affected by water deficit [1]. Although the concentrations of some amino acids decline during water stress, there is an overall increase in the concentration of soluble nitrogenous compounds [2]. Water stress leads to a rapid and extensive accumulation of proline which varies considerably between different genotypes. A decrease in nitrate reductase in corn and barley [1], and an increase in ribonuclease activity [4] with moisture stress has been observed. An earlier study [5] showed differences in peroxidase isoenzymes in rice varieties differing in their tolerance to drought. However, the information on changes in various enzymes in genotypes differing in their tolerance to drought is lacking. Therefore in the present study the effect of moisture stress on peroxidase, ribonuclease, nitrate reductase, protease and proline accumulation in TKM-1 a drought tolerant and Improved Sabarmati (I.S.) a drought susceptible rice variety has been studied.

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RESULTS AND DISCUSSION

Peroxidase

Sp. act. of peroxidase in TKM-1 and I.S. in 72 hr imbibed seeds and 8-day-old shoot and root at different water stress is shown in Table 1. At 72 hr imbibition sp. act. of peroxidase decreased with increasing moisture stress both in TKM-1 and I.S. The decrease in I.S. was 74% while in TKM-1 it was only 37%. Peroxidase activity did not change much in the shoots of either variety. In roots of TKM-1 also peroxidase activity increased with moisture stress while in I.S. roots it decreased. These observations further support the greater decrease in peroxidase isoenzyme bands [5] and their intensities in I.S. as compared to TKM-1

RNase activity

Since moisture stress is known to affect RNA metabolism, RNase activity in TKM-1 and I.S. was studied in 72 hr imbibed seeds and in 8-day-old shoots and roots at different water stress levels and the results are presented in Tables 2 and 3. Considerable differences in RNase activity between TKM-1 and I.S. were observed. At 72 hr imbibition the sp. act. of RNase decreased with increasing water stress. However, the decrease in sp. act. of RNase in I.S. was 71% while in TKM-1 it was 55% (Table 2). Sp. act. of RNase in TKM-1 at −15 bars osmotic potential (ψ) was considerably higher compared to I.S. even

Table 1. Peroxidase activity (ΔA/mg protein) in TKM-1 and I.S. at different moisture stress

Treatment	TKM-1			I.S.		
	72 hr imbibition	Shoot 8 day	Root 8 day	72 hr imbibition	Shoot 8 day	Root 8 day
Control*	39.8	23.9	147	24.7	31.1	191
−2.5 bars (Mannitol)	34.5	29.2	200	15.3	33.7	177
−5.0 bars (Mannitol)	26.3	31.1	199	11.3	32.1	161
−7.5 bars (Mannitol)	25.2	30.5	204	6.3	32.9	150

* Water as medium.

Table 2. RNase activity in TKM-1 and I.S. at 72 hr imbibition with water stress

Osmotic potential by PEG (bars)	TKM-1		I.S.	
	per mg Protein	Ratio RNase A/B	per mg Protein	Ratio RNase A/B
Control	25.4	0.89	25.2	0.65
−3	20.8	0.76	20.6	0.65
−5	18.9	0.76	13.9	0.72
−10	11.9	0.74	9.9	0.81
−15	11.3	0.72	7.4	0.8

Table 4. Protease activity (per mg protein) in 72 hr imbibed seeds and 8-day-old shoots of TKM-1 and I.S. with water stress

Osmotic potential createdby PEG (bars)	72 hr imbibed seed		Shoots	
	TKM-1	I.S.	TKM-1	I.S.
Control	0.373	Traces	0.519	0.208
−5	0.215	Nil	0.611	0.307
−10	0.073	Nil	0.763	0.216
−15	0.050	Nil	0.671	0.168

though the RNase activity in seeds of both the varieties imbibed in water was nearly the same. The ratio of RNase A/B decreased in TKM-1 and increased in I.S.

Sp. act. of RNase in shoots increased in I.S. at −5 bar ψ while in TKM-1 it remained more or less the same. Further increase in water stress resulted in decrease in enzyme activity in I.S. shoots. The ratio of RNase A/B did not change much during water stress. RNase sp. act. decreased in TKM-1 roots with increase in water stress while in I.S. it showed an increase up to −10 bars ψ and a slight decrease thereafter. The RNase A/B ratio decreased at −15 bars ψ in TKM-1 roots while in I.S. it remained more or less the same.

Protease

Effect of water stress on protease activity in TKM-1 and I.S. shoots and 72 hr imbibed seeds is shown in Table 4. Protease activity was higher in TKM-1 as compared to I.S. both in 72 hr imbibed seeds and 8-day-old shoots. At 72 hr imbibition only trace of protease activity was detected in I.S. In TKM-1 protease activity decreased with increase in water stress at 72 hr imbibition. On the other hand protease activity in shoots of TKM-1 increased appreciably with increase in water stress up to −10 bars ψ thereafter it decreased slightly. The sp. act. of protease in I.S. shoots decreased after −5 bars ψ. The sp. act. of protease in TKM-1 shoots was considerably higher than I.S. shoots at all the moisture stress levels. At −15 bars ψ the protease activity in I.S. was 25% of the activity in TKM-1 shoots. The roots of both the varieties did not show any protease activity. Considerably higher protease activity in shoots of TKM-1, the drought tolerant variety compared to I.S. might be of significance in drought adaptability since seedlings have to adapt during water stress and adjust the metabolism in such a way that the energy requirements are minimum. Since the soluble protein content does not decrease but increases during water stress [5]. the increase in protease activity might be responsible for selectively degrading some storage proteins.

Nitrate reductase

Nitrate reductase activity in shoots of TKM-1 and I.S. at different water stress levels is shown in Table 5. In TKM-1 shoots nitrate reductase activity increased up to −2 bars ψ and thereafter decreased slightly while in I.S. shoots a 20% decrease in sp. act. occurred at −2 bars ψ. Decrease in nitrate reductase with water stress in corn leaves [6, 7] and barley [8] has been observed due to the decreased rate of enzyme synthesis. Increase in nitrate reductase with water stress in drought tolerant variety TKM-1 indicates higher capacity for enzyme synthesis as compared to I.S.

Proline

Free proline contents in shoots of TKM-1 and I.S. at different water stress levels are shown in Table 6. In both rice varieties, free proline content increased with increas-

Table 5. Nitrate reductase activity (nmol of NO_2 formed/30 min) of shoots of TKM-1 and I.S. with water stress

Osmotic potential by PEG (bars)	TKM-1 per mg protein	I.S. per mg protein
Control	12.3	14.4
−1	14.1	15
−2	15.4	11.4
−3	14.1	12.7

Table 3. RNase activity in 8-day-old shoots and roots of TKM-1 and I.S. with water stress

Osmotic potential by PEG (bars)	Shoot				Root			
	TKM-1		I.S.		TKM-1		I.S.	
	Activity/ mg protein	Ratio RNase A/B	Activity/ mg protein	Ratio RNase A/B	Activity/ mg protein	Ratio RNase A/B	Activity/ mg protein	Ratio RNase A/B
Control	28.1	0.8	15.9	0.8	110	1.1	111	1.1
−5	27.7	0.8	33.8	0.71	93.5	1	132	1
−10	26.6	0.75	25.3	0.78	88.5	0.98	151	1
−15	24.8	0.85	18.8	0.72	80.6	0.76	144	1

Table 6. Free proline contents of shoots of TKM-1 and I.S. with water stress

Osmotic potential by PEG (bars)	TKM-1 μg/g dry wt	I.S. μg/g dry wt
Control	250	755
−5	990	900
−10	1350	910
−15	910	900

ing water stress, but the increase was more marked in TKM-1 as compared to I.S. In TKM-1 more than a 5-fold increase in proline content was observed whereas in I.S. it was only 1.2 fold at −10 bars ψ. The absolute level of proline in I.S. was 3 times that of TKM-1. At −5 bars ψ proline concentration in TKM-1 increased by *ca* 4 fold while in I.S. the increase was only 20%. These observations show that although the absolute level of proline in TKM-1 is lower, a rapid accumulation of proline occurs in it when subjected to water stress. It is further evident that proline itself is not involved in drought tolerance since proline concentration in I.S. even at −15 bars ψ was equal to TKM-1. Increase in proline content in rice with decreasing water potential has been observed [9]. Increase in proline could occur either due to fresh synthesis or from breakdown of proline rich proteins during stress. However, in the present investigation *de novo* synthesis must account for an increase in non-protein proline as soluble protein content did not decrease during water stress. This is further supported by the observation that the primary effect of wilting in excised bean leaves which leads to the accumulation of proline was to decrease protein synthesis and increase proline formation [10]. A close correlation between free proline accumulation in the tissues of plants and their resistance to drought has also been observed [1, 3, 11]. The differential response in proline accumulation observed in the present study between TKM-1 and I.S. differing in their adaptability to drought indicates that the proline accumulation potential of varieties varies with the adaptability to drought conditions.

The drought tolerant variety is thus characterized by higher peroxidase and protease activities at 72 hr imbibition, and greater capacity for free proline accumulation in shoots with water stress as compared to the susceptible variety.

EXPERIMENTAL

Seeds were sterilized and grown in an illuminated germinating chamber at 20° in the presence of solns of different osmotic potential according to ref. [5].

Extraction of soluble protein. Germinating seeds, shoots or roots were washed with H_2O, blotted and ground in a chilled pestle and mortar with 50 mM Tris–HCl buffer (pH 7.6) for peroxidase and RNase. For protease, nitrate reductase 0.1 M Pi buffer (pH 7.5) containing 1 mM cysteine was used. All operations unless otherwise stated were carried out at 4°. The homogenate was centrifuged at 10000 *g* for 20 min at 0° for enzyme assay. Protein was estimated by the method of ref. [12]. Peroxidase activity was assayed with slight modification of the method used by ref. [13]. The activity has been expressed as change in *A* per min per mg protein at 460 nm. Ribonuclease assay was done at pH 5.2, 5.8 and 6.8 according to ref. [14]. The results are expressed as enzyme units. One unit of RNase activity corresponding to the amount of enzyme which causes an increase of 1 in *A* over an enzyme blank. Nitrate reductase activity was assayed according to ref. [15] with the modification that the reaction was stopped by adding 100 μl of M Zn acetate and 1.9 ml of 70% EtOH. Protease activity was assayed according to ref. [16]. Free proline in seeds was determined according to ref. [17]. The results are the means of at least duplicates which agreed closely.

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