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EFFECT OF WATER STRESS ON THE ENZYMES OF NITRATE METABOLISM IN TWO *BRASSICA* SPECIES*

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Key Word Index—*Brassica juncea*; *B. campestris*; Cruciferae; transaminase; nitrate reductase; glutamate dehydrogenase; proline; water stress; polyethylene glycol-6000.

Abstract—*Brassica juncea* (drought susceptible) and *B. campestris* (drought tolerant) were germinated under simulated water stress created by polyethylene glycol (MW 6000). The two species showed characteristic differences in dry weight, nitrate reductase, aspartate amino transferase, alanine aminotransferase, glutamate dehydrogenase and free proline accumulation in the embryo axis under water stress. Stress resulted in the decreased activities of these enzymes and the decrease was more in *B. juncea* than in *B. campestris*. In both species, protein content was higher under stress. In *B. juncea*, a 12-fold increase in free proline occurred as compared to a 7-fold increase in *B. campestris* at −6 atm osmotic potential.

INTRODUCTION

Water stress has been shown to affect all plant processes [1] and because of great variations in susceptibility to drying of different plants, there have been many attempts to associate these differences with particular subcellular features including enzymes [1–4]. It has been suggested that a more favourable balance between synthesis and breakdown is maintained by drought tolerant variety/species [5]. Unfortunately very little work has been done on enzymes of nitrate metabolism, except for a decrease in nitrate reductase in different crops that has been reported [1, 6, 7]. Contrary to this, an increase in nitrate reductase under mild stress has been reported in rice seedlings [3]. However, the information on changes in various enzymes of nitrate metabolism in species differing in their relative tolerance to drought is lacking. Therefore, in the present work the effect of simulated water stress on nitrate reductase (NR), glutamate dehydrogenase (GDH), aspartate amino transferase (GOT), alanine aminotransferase (GPT), protein and free proline accumulation in *B. juncea* (L.) Czern & Coss a drought susceptible and *B. campestris* L. a drought tolerant species of *Brassica* has been studied during germination.

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RESULTS AND DISCUSSION

Results presented in Table 1 showed that the specific activity of NR in the 120-hr-old embryo axis decreased markedly and consistently with the increase in the level of water stress. The specific activities of GDH, GOT, and GPT also decreased in both the species with the increase in water stress (Table 1). The maximum reduction (90%) was observed in NR activity followed by GPT, whereas least reduction was observed in GOT and GDH. The overall activity of NR and GDH was more in *B. campestris* than in *B. juncea*, whereas the reverse was true for transaminases. The decrease in NR under water stress has been reported in different plants [1, 6–8]. However, an increase in NR activity was reported in rice seedlings grown under mild water stress [3].

As these enzymes are involved in the synthesis of protein amino acids, the decrease in the activity of these enzymes would result in the reduced synthesis of protein and thus would express itself in reduced seedling growth. Under water stress, the reduction in the activities of various enzymes studied was more in *B. juncea* than in *B. campestris*, which correlates well with the relative growth of the two species under these conditions (Table 2). Under stress, the dry weight of the embryo axis was more in *B. campestris* than in *B. juncea* (Table 2). It has been shown that the former is entirely cultivated on unirrigated land, while the latter is best grown under irrigated conditions [9].

Water stress has been shown to reduce the protein content [10, 11]. However, results presented in Table 2

Table 1. Activities of enzymes in the embryo axis of *B. juncea* and *B. campestris* at 120 hr after sowing under water stress

Osmotic potential (atm)	Enzyme units/mg protein*							
	B. juncea				*B. campestris*			
	NR	GDH	GOT	GPT	NR	GDH	GOT	GPT
Control	2.73	26.53	3.91	1.96	3.64	36.46	2.97	1.86
−2	1.78	11.97	2.06	0.78	2.13	18.68	1.89	0.95
−4	1.02	10.53	1.64	0.66	1.09	17.17	2.50	0.45
−6	0.35	11.53	1.44	0.21	0.50	18.50	1.88	0.29

* For enzyme key, see text.

Table 2. Effect of water stress on dry wt, protein and free proline content in the embryo axis of *B. juncea* and *B. campestris* at 120 hr after sowing

Osmotic potential (atm)	*B. juncea* Dry wt (mg/10 seedlings)	Protein (mg/g dry wt)	Proline (mg/g dry wt)	*B. campestris* Dry wt (mg/10 seedlings)	Protein (mg/g dry wt)	Proline (mg/g dry wt)
Control	11.3	40.67	3.07	16.0	43.71	3.03
−2	8.0	46.53	10.89	15.5	46.59	4.82
−4	5.5	46.24	16.59	9.0	45.44	13.42
−6	4.8	80.73	37.52	8.0	69.03	20.60

showed an increase in the protein content under water stress. The increase was more under higher stress and less under low stress. The increase at −6 atm was more in *B. juncea* than in *B. campestris*. The increase in protein under stress has also been reported earlier [2]. This increased protein content would probably result from relatively greater numbers of cells in a unit tissue weight under water stress, since cell elongation rather than cell division is reduced more under water stress [1].

A close correlation between free proline accumulation in the tissues of plants and their resistance to drought has been observed [3, 12]. In contrast, results presented in Table 2 showed greater accumulation of free proline in the drought susceptible (*B. juncea*) than relatively drought tolerant (*B. campestris*) species. Similar greater accumulation of free proline in susceptible than in tolerant varieties of barley has also been reported [13]. This increase in proline could occur either due to *de novo* synthesis or from breakdown of proline rich proteins during stress. However, in the present investigation *de novo* synthesis must account for an increase in proline, as soluble protein content did not decrease but increased under water stress (Table 2). It can be concluded that proline accumulation serves as a good index of the degree of stress but is not necessarily an index of drought resistance.

EXPERIMENTAL

Seeds of *B. juncea* and *B. campestris* were surface-sterilized with $HgCl_2$ and grown in a germinating chamber at 25° in the presence of aq. solns of different osmotic potentials prepared as described earlier [4].

Extraction of soluble protein. After 96 and 120 hr of sowing, seeds were washed with distilled water, blotted and ground in a chilled pestle and mortar with 0.1 M Tris–HCl buffer (pH 7.6) containing 10^{-4} M cysteine and 10^{-4} M EDTA. All operations unless otherwise stated were carried out at 4°. The homogenate was centrifuged at 12000 *g* for 30 min. Protein was estimated according to ref. [14]. GOT and GPT were assayed according to ref. [15]. Activities were expressed as mM of pyruvate liberated in 30 min. The activity of GDH was estimated according to [16] and expressed in terms of change in 0.01 A_{340} per min. The *in vivo* estimation of the nitrate reductase activity in intact tissue was done according to [17]. Activity was expressed as μg NO_2 liberated under the experimental conditions. Proline was extracted and estimated as described earlier [18]. The results are the means of at least duplicates which agreed closely. The data of only 120-hr-old seedlings were presented as the data of 96-hr-old seedlings were similar to that of 120-hr-old seedlings.

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