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EFFECT OF SALT STRESS ON POLYAMINE METABOLISM IN *BRASSICA CAMPESTRIS*

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Key Word Index—*Brassica campestris*; Cruciferae; salt; polyamine; seedling; stress; cultivar.

Abstract—Polyamines (PAs) and their major metabolic enzymes were studied under different modes of salinity stress in *Brassica*. Long stress caused only small changes in PA level, arginine (EC 4.1.1.19) and ornithine decarboxylases (EC 4.1.1.17) and polyamine oxidase (EC 1.5.3.3) activity. However, short stress increased PA level and enzyme activities. Diamine oxidase (EC 1.4.3.6) activity, unlike other PA metabolic enzymes, increased significantly during long stress.

INTRODUCTION

In recent years, much attention has been paid to the involvement of polyamine (PA) in plant growth, differentiation, aging, senescence and in response to stress [1–3]. Although the precise mechanisms of action for the PAs remain elusive, they are undoubtedly important cellular constituents [4, 5]. The physico-chemical characteristics of the PAs support macromolecular interactions with various modes of complex formation. In a variety of experimental systems, increased levels of free PAs are often paralleled by an increase in the activity of arginine decarboxylase (ADC), while ornithine decarboxylase (ODC) appears to be unaffected [6, 7]. The activities of diamine oxidase (DAO) and polyamine oxidase (PAO) in relation to stress have not been well studied. The present work reports the different responses of PAs and their metabolic enzymes under long and short term NaCl stress in *Brassica* seedlings.

RESULTS AND DISCUSSION

The main feature of our investigation is the accumulation of PAs under short salinity stress. The different impact of long stress and short stress was reflected in PA accumulation (Fig. 1) and activities of the major metabolic enzymes (Fig. 2). From the study of different physiological parameters, cultivar B-9 was found to be relatively salt sensitive and cultivar B-54 was salt tolerant [8]. The endogenous PA content (Fig. 1c) and ADC activity (Fig. 2a) were higher in cv B-54, while DAO activity was slightly increased in cv B-9 (Fig. 2c). In control seedlings, B-54 has the greatest amount of spermidine (spd).

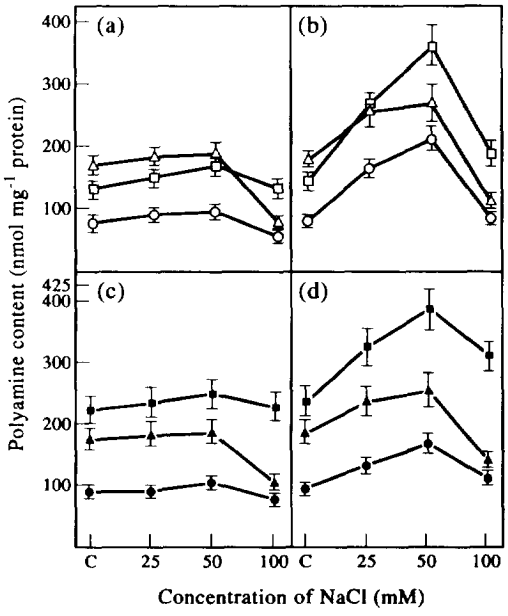

Fig. 1. Effect of NaCl on PA content of B-9 cultivar [put (△), spd (□) and spn (○)] under (a) long stress and (b) short stress and of B-54 cultivar [put (▲), spd (■) and spn (●)] under (c) long stress and (d) short stress of 72 hr old *Brassica campestris* seedling. Data are means of three replicates in each of three separate experiments + s.e.

Long stress has least influence on PA level in both B-9 and B-54 (Fig. 1a,c). B-9 showed maximum 20% increase in PA content of which the rise of spd and spermine (spn) were 28% and 27%, respectively, at 50 mM NaCl. The

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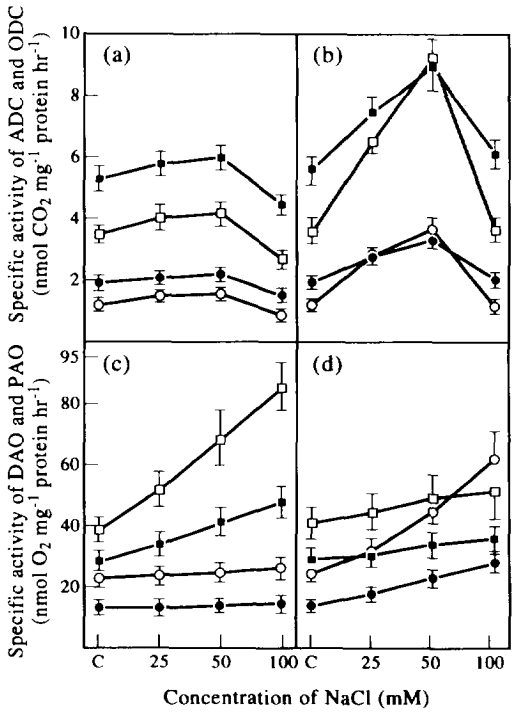

Fig. 2. Effect of NaCl on the specific activity of ADC [cv B-9 (□) and cv B-54 (■)] and ODC [cv B-9 (○) and cv B-54 (●)] under (a) long stress and (b) short stress and change in specific activity of DAO [cv B-9 (□) and cv B-54 (■)] and PAO [cv B-9 (○) and cv B-54 (●)] under (c) long stress and (d) short stress of 72 hr old *B. campestris* seedling. Data are means of three replicates in each of three separate experiments + s.e.

slight increase in total as well as individual PA content was observed in B-54 also at 50 mM NaCl. Short stress however, had a profound effect on PA level (Fig. 1b,d). The total PA level increased 1.6- and 2.1-fold at 25 mM and 50 mM NaCl, respectively, in B-9 (Fig. 1b), while in B-54 (Fig. 1d) it was 1.3- and 1.6-fold. At 100 mM NaCl, putrescine (put) titre decreased significantly in both long and short stress in both cultivars. However, spd level was always higher than put under short stress. The individual PAs put, spd and spn increased 1.5-, 2.5-, 2.6-fold and 1.3-, 1.6-, 1.7-fold in B-9 and B-54, respectively, at 50 mM NaCl. Total PA level decreased in both cultivars, but spd level slightly increased in B-54 at 100 mM NaCl. Accumulation of put, a common feature, under a wide range of stress conditions has been well documented in ref. [9]. It has been postulated that production of spd or other high M_r PAs may be a key factor in plant cellular protection against osmotic shock [10, 11]. Increased level of spd and spn under short salinity stress had been reported in NaCl resistant cell lines from *Nicotiana sylvestris* [12] and also in a salt tolerant rice cultivar [13]. The two drought tolerant populations of alfalfa showed increased level of spd and spn which ultimately led to the formation of the long chain uncommon PAs, not spd and not spn, under osmotically stressed conditions [4]. Possibly during the period of metabolic adaptation to salinity, spd and spn stabilize membrane systems [13].

ADC and ODC activities were almost unaltered during long stress (Fig. 2a). However, response was quite pronounced during short stress (Fig. 2b) where ADC and ODC activities increased 1.8- and 2.5-fold, respectively, at 25 mM, and 2.2- and 3-fold at 50 mM NaCl in B-9. In B-54 the increase was 1.3- and 1.6-fold as well as 1.4- and 1.7-fold at the respective NaCl concentration. One hundred mM NaCl was almost as deleterious to biosynthetic enzymes in both cultivars. Stress induced activation of ADC and ODC, as reported in mung bean in an organ specific manner [14], suggests coordinated changes in PAs in the whole plant. Increased ADC and ODC activities were also observed in cereal leaves [10]. Response to salinity stress was greater in ODC than ADC in *Brassica* although ADC was considered to be a general stress enzyme in cereal [15].

Different modes of salinity stress have differential impacts on PA biodegradative enzymes in *Brassica*. DAO activity was much influenced during long stress (Fig. 2c), while PAO activity increased considerably during short stress (Fig. 2d). DAO activity increased 1.8- and 2.2-fold at 50 mM and 100 mM NaCl, respectively in B-9, and it was 1.5- and 1.8-fold in B-54 during long stress. On the other hand, PAO activity increased 1.8- and 2.5-fold in B-9, and 1.6- and 2-fold in B-54 at 50 mM and 100 mM NaCl during short stress. Stress induced inhibition of DAO and PAO was reported in ref. [15]. PAO activity was largely stimulated in the root meristem tissues of drought tolerant alfalfa under osmotic stress [4].

EXPERIMENTAL

Seeds of *Brassica campestris* cv B-9 and B-54 were collected from Bose Institute experimental farm at Madhyamgram, West Bengal. After surface sterilization with 0.1% $HgCl_2$, NaCl stress was applied in two ways: (a) for 'long term' stress, imbibition (2 hr) and germination of the seeds were carried out in Petri dishes in different salt concn, i.e. 25 mM, 50 mM and 100 mM at 22 ± 2° for 72 hr; (b) for 'short term' stress, 72 hr control seedlings (germinated in H_2O) were salinized with respective NaCl soln for 6 hr. In every case a control was taken in H_2O.

PA estimation. PA content was determined after dansylation according to ref. [17] with some modifications. Sample (19) was homogenized with 3 vols of 5% (w/v) TCA and centrifuged at 10000 *g* for 15 min. The supernatant was extracted with Et_2O (× 3) to remove TCA. To 0.1 ml of aq. extract, were added 0.1 ml satd $NaHCO_3$ and 0.2 ml dansyl chloride (30 mg ml^{-1} in Me_2CO), and the mixt. was kept for 18 hr in the dark at 25°. The dansylated amines extracted with C_6H_5Me were loaded on a HPTLC plate of silica gel 60 with concn zone (Merck) using C_6H_{12}–EtOAc (3:2) as the developing solvent. The spots corresponding to the authentic PAs were eluted with Me_2CO and the fluorescence intensities were measured with excitation and emission at 360 and 506 nm, respectively.

ADC and ODC activities. Determined by measuring the non-artifactual $^{14}CO_2$ from L-[U-^{14}C] arginine (sp. act.

180 mCi mmol^{-1}) and L-[1-^{14}C] ornithine (sp. act. 61 mCi mmol^{-1}), respectively, following a modification of the method of ref. [18]. The extraction and assay medium consisted of 10 mM kPi buffer (pH 8), 0.1 mM pyridoxal-5'-phosphate, 1 mM DTT and 2 mM Na-EDTA. For ADC, artifactual decarboxylation was estimated by adding 50 μl of 100 mM DFMA to the reaction mixt. before ADC assay. In every case non-enzymatic decarboxylation was 5–10% of experimental values.

DAO and PAO activities. Estimated in Hansatech oxigraph equipped with Clark electrode according to refs [19] and [20], respectively.

Protein estimation. Protein was estimated as in ref. [21].

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