BIOLOGIA PLANTARUM 44 (4): 607-609, 2001

BRIEF COMMUNICATION

Effects of NaCl and proline on polyphenol oxidase activity in bean seedlings

Y. DEMİR* and İ. KOCAÇALIŞKAN**

*Atatürk Üniversitesi, K.K. Eğitim Fakültesi, Biyoloji Bölümü, TK-25240 Erzurum, Türkey**
*Dumlupınar Üniversitesi, Fen-Edebiyat Fakültesi Biyoloji Bölümü, TK-43100 Kütahya, Türkey***

Abstract

In this work, the effects of NaCl (0, 50, 100, and 150 mM), proline (0, 5 and 10 mM) and NaCl + proline in combinations on activity of polyphenol oxidase (PPO; E.C. 1.10.3.1) and soluble protein content have been investigated in the root, stem and leaf tissues of bean (*Phaseolus vulgaris* L.) seedlings grown in embryo culture. PPO activities were higher in all the tissues treated with NaCl, proline and NaCl + proline combinations those that of the control tissues. The protein content was very high in tissues exposed to proline and NaCl + proline combination, but NaCl alone decreased protein contents in root and leaf tissues. The results suggest that proline may play a role as an enzyme-stabilizing agent in salt stress.

Additional key words: embryo culture, *Phaseolus vulgaris*, salt stress.

Salinity is serious problem for agricultural productivity in many parts of the world. To date, studies on salinity have focussed predominantly on germination percentage and growth of seedlings and plants. There are several studies on the effect of salinity on the enzymes involved in seed reserve mobilization such as amylase (Ramagapol 1987), protease (Prisco *et al.* 1981), and RNase (Sheoran and Garg 1978). A number of oxidation systems other than the major oxidative phosphorylation in the mitochondria have been reported in seeds and seedlings. They may interfere with normal respiration or they may play a role in the recycling of reducing equivalents. One of them polyphenol oxidase which oxidize some phenols to quinones is important (Gomes Filho and Sodek 1988). This enzyme has been studied in a number of plants in relation to diseases, wounding (Maxwell and Bateman 1967), hormonal regulation (Hulme and Rhodes 1971), enzymatic browning (Jennings and Duffus 1977), and germination (Kocaçalışkan and Özbay 1987, Kocaçalışkan *et al.* 1995). However, information concerning its affection by salt stress is insufficient.

Many researchers have ascribed to proline a positive role under stress. In contrast, Hanson *et al.* (1977) considered proline accumulation to be a symptom of damage. According to Stewart and Lee (1974), proline is osmotic agent. Other researchers have suggested that proline is a source of energy, carbon and nitrogen for the recovering tissues (Blum and Ebercon 1976). Proline can protect cells against damage induced by ultraviolet radiation (Demir 2000).

We have not encountered any report on the response of PPO to NaCl and proline in the bean seedlings. Therefore, it was interesting to study whether proline stabilize the enzyme or not. For this aim, dwarf bean was chosen as experiment material, since it is known to be sensitive for salinity.

Seeds of dwarf bean (*Phaseolus vulgaris* L. cv. Kızılhaç) were surface sterilized with 1 % sodium hypochlorite. Embryos of the seeds were excised and cultured in the basal medium of Murashige and Skoog (1962; MS) without hormones and supplemented with NaCl (0, 50, 100, and 150 mM) and proline (0, 5 and 10 mM). Embryos were grown for 7 d at 14-h photoperiod, irradiance of 222 μmol m^{-2} s^{-1} (400-700 nm), and temperature of 24 ± 1 °C).

Fresh tissue samples (0.5 g) from root, stem and leaf of the seedlings were homogenized with 5 cm^3 cold 0.05 M sodium phosphate buffer (pH 6.5) for 2 min and

Received 14 November 2000, *accepted* 27 February 2001.
Abbreviations: DOPA - dihydroxyphenylalanine; PPO - polyphenol oxidase.
Fax: (+90) 442 2184172, e-mail: serdengecti@yahoo.com

filtered, then centrifuged at 20 000 *g* for 15 min at 4 °C. Then the enzyme activities were immediately determined spectrophotometrically as described by Jennings and Duffus (1977). 3,4-dihydroxyphenylalanine (DOPA; 10 mM), prepared in 0.1 M (pH 6.5) sodium phosphate buffer were used as a substrate. The reaction mixture containing 2.8 cm^3 of substrate solution and 0.2 cm^3 of crude extract was incubated at room temperature for 2 min. The absorbances of the mixture were measured spectrophotometerically (*Shimadzu UV 1208* spectrophotometer, Japan) at 420 nm. The mixture without crude extract served as blank. The amount of soluble protein in the enzyme extraction was estimated according to Bradford (1976) using bovine serum albumin as standard.

In the roots, stems and leaves treated with NaCl, PPO activity gradually increased as NaCl concentrations increased (Fig. 1). A significant correlation between NaCl concentration and PPO activity (r = 0.91) was obtained. The responses of PPO to NaCl were different in roots, stems and leaves. Similar results were obtained when responses of peroxidase and catechol oxidase to NaCl were followed (Gaurab *et al.* 1996, Converso *et al.* 1997). Proline (5 and 10 mM) applied alone or in combination with NaCl increased PPO activity in all plant parts (Fig. 1).

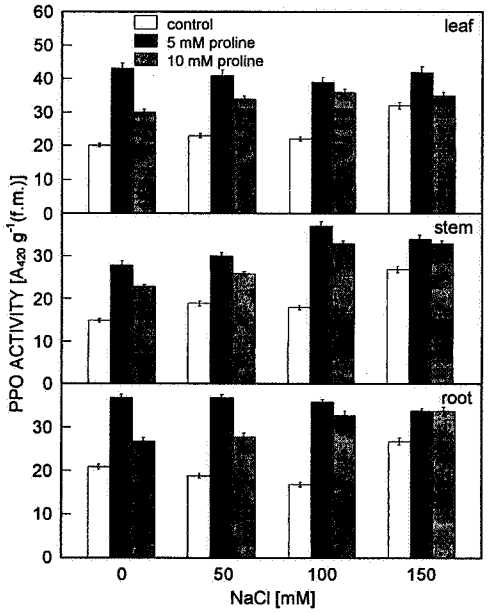

Fig. 1. Effects of NaCl and proline in different concentrations on PPO activities in leaf, stem and root tissues of 7-d-old bean seedlings grown in embryo culture. Means ± standard errors for triplicate samples.

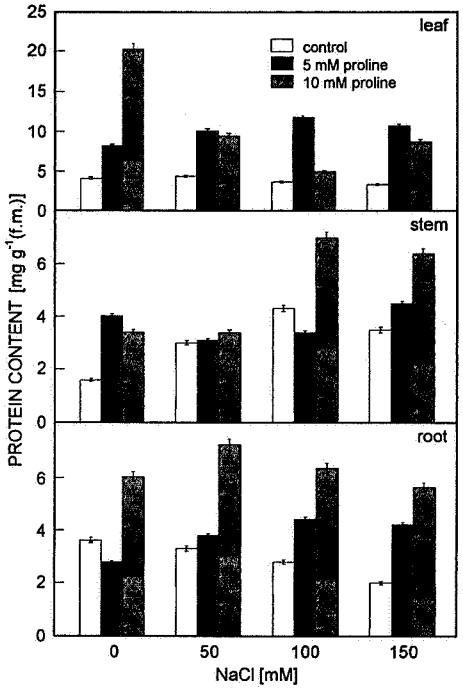

Fig. 2. Effects of NaCl and proline in different concentrations protein content in root, stem and leaf tissues of 7-d-old bean seedlings grown in embryo culture. Means ± standard errors for triplicate samples.

In leaves and roots, but not in stems, soluble protein contents decreased with increased NaCl concentration (negative correlation; r = -0.186). The highest soluble protein content was recorded in the leaf tissue (Fig. 2). Other scientists have shown that salinity inhibits protein synthesis, delay RNA synthesis (*e.g.* Marie *et al.* 1989), and accelerates degradation of RNA (*e.g.* Sheoran and Garg 1978). Therefore, the decreasing of protein content by NaCl applications may be due to one of the mechanisms mentioned above. Proline enhanced the protein contents in all tissues, more effective was 10 mM proline. Interaction between proline and polyamines and stabilizing effects of proline on enzymes and membranes were reported (*e.g.* Tiburcio *et al.* 1993, Miranda and Loyola-Vargas 1994). Thus, it may also protect the cell against damage induced by free radicals which may increase under salt stress. NaCl treatment increased the activities of the antioxidative enzymes, catalase and glutathione reductase (Lechno *et al.* 1997).

From the result obtained, it seems that proline might play a role as an enzyme-stabilizing agent in salt stress.

References

Blum, A., Ebercon A.: Genotypic responses in sorghum to drought stress. III. Free proline accumulation and drought resistance. - Crop Sci. **16**: 428-431, 1976.

Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein-dye binding. - Anal. Biochem. **72**: 248-254, 1976.

Converso, D.A., Fernandez, M.E., Tomaro, M.L.: Effect of

development and salt stress on the activity and expression of wheat peroxidases. - Phyton **61**: 147-154, 1997.

Demir, Y.: Growth and proline content of germinating wheat genotypes under ultraviolet light. - Turk. J. Bot. **24**: 67-70, 2000.

Gaurab, G., Sangita, B., Mukherjee, B., Sukumar, G., Gangopadhyay, G., Basu, S., Gupta, S.: Salinity-induced changes on peroxidase in *Nicotiana tabacum* (var. Jayasri) callus cultures. - Indian J. Plant Physiol. **1**: 247-250, 1996.

Gomes Filho, E., Sodek, L.: Effect of salinity on ribonuclease activity of *Vigna unguiculata* cotyledons during germination. - J. Plant Physiol. **132**: 307-311, 1988.

Hanson, A.D., Nelson, C.E., Eversen, E.H.: Evolution of free proline accumulation as an index of drought resistance using two contrasting barley cultivars. - Crop Sci. **17**: 720-726, 1977.

Hulme, A.C., Rhodes, M.J.C.: Pome Fruits. The Biochemistry of Their Products. - Academic Press, London 1971.

Jennings, P.H., Duffus, C.M.: Effect of giberellic acid on polyphenol oxidase activity in de-embryonated wheat and barley grains. - New Phytol. **78**: 383-389, 1977.

Kocaçalışkan, I., Demir, Y., Kabar, K.A.: Study on polyphenol oxidase activity during seed germination. - Phyton **35**: 37-43, 1995.

Kocaçalışkan, İ., Özbay, O.: [Changes in enzymatic browning and related factors during development of potato.] - Doga Turk. Biyol. **11**: 124-129, 1987. [In Turk.]

Lechno, S., Zamski, E., Tel Or, E.: Salt stress-induced responses in cucumber plants. - J. Plant Physiol. **150**: 206-211, 1997.

Marie, F.R., Jar, P.S., Nikos, B., Jean, P.B., Robert, E.: Effect of salinity on ribonucleolytic activities in glycophytic and halophytic plant species. - J. Plant Physiol. **133**: 738-742, 1989.

Maxwell, D.P., Bateman, D.F.: Changes in the activity of some oxidases in extracts of rhizoctonia infected bean hypocotyles in relation to lesion maturation. - Phytopathology **57**: 132-135, 1967.

Miranda, H.M.deL., Loyola-Vargas, V.M.: Glutamate dehydrogenase and glutamine synthase activities in maize under water and salt stress. - Phyton **56**: 7-15, 1994.

Murashige, T., Skoog, F.: A revised medium for rapid growth and bioassays with tobacco tissue cultures. - Physiol Plant. **15**: 473-497, 1962.

Prisco, J.T., Enear Filho, J. Gomes Filho, E.: Effect of NaCl salinity on cotyledon starch mobilization during germination of *Vigna unguiculata* L. Walp seeds. - Rev. brasil. Bot. **4**: 63-71, 1981.

Ramagapol, S.: Salinity stress induced tissue-specific proteins in barley seedlings. - Plant Physiol. **84**: 324-331, 1987.

Ramagapol, S.: Regulation of protein synthesis in root, shoot and embryonic tissues of germinating barley during salinity stress. - Plant Cell Environ. **11**: 501-515, 1988.

Sheoran, I.S., Garg, O.P. Effect of salinity on the activities of RNase, DNase, and protease during germination and early seedlings growth of mung bean. - Physiol. Plant. **44**: 171-174, 1978.

Stewart, G.R., Lee, J.A. The role of proline accumulation in halophytes. - Planta **120**: 279-289, 1974.

Tiburcio, A.F., Campos, J.L., Figueras, X., Besford, R.T.: Recent advances in the understanding of polyamine functions during plant development. - Plant Growth Regul. **12**: 331-340, 1993.