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BRIEF COMMUNICATION

# Effect of NaCl and proline on bean seedlings cultured *in vitro*

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## Abstract

Effects of NaCl (150 mM), proline (10 mM) and their combination on growth and contents of chlorophyll, proline and protein of bean (*Phaseolus vulgaris* cv. Kızılhaç) seedlings *in vitro* were investigated. NaCl decreased seedling growth. Proline added to control seedlings did not change seedling growth but decreased chlorophyll and increased protein contents. When proline added to NaCl-treated seedlings growth was increased in comparison with NaCl-treated only. Thus, proline alleviated salinity stress in bean seedlings.

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

Salinity is one of the major stress for plants, which reduced their growth and yield. Plants are stressed in two ways in high salt environment: by the increase in osmotic potential of the rooting medium as a result of high solute content, and by the toxic effects of high concentrations of the ions. Proline accumulation in plants subjected to salt stress has been indicated in several reports (Stewart and Lee 1974, Tıpırdamaz and Karakullukçu 1993). There are also several reports on the role of proline in salt tolerance of plants; by maintaining osmoregulation (Wyn-Jones and Storey 1978), stabilizing proteins (Schobert and Tschesche 1978), protecting cell membrane and cytoplasmic enzymes (Ahmad *et al.* 1982). Even proline accumulation has been offered as a measure of salt tolerance (Hanson and Hitz 1982). However, some researchers are claim that proline accumulation is a coincidental result of metabolic irregularities created by the salt stress, and therefore it has no adaptive value (Fukutoku and Yamada 1984, Tıpırdamaz and Çakırlar 1990). Thus physiological role of proline in plants under salt stress is still uncertain.

Bean is known as a non-tolerant species to salt stress. We have not encountered any report about proline effect on salt stress in *Phaseolus vulgaris*. Therefore this research was conducted to investigate the effect of exogenously applied NaCl and proline on growth of bean seedlings *in vitro*.

Seeds of bean (*Phaseolus vulgaris* L. cv. Kızılhaç) were surface sterilized by soaking in 1 % sodium-hypochloride for 10 min and washed with distilled water. Embryos of the seeds were excised and transferred into the glass jars containing sterile Murashige and Skoog (1962) medium, without hormones. The pH of the medium was adjusted to 5.7 with KOH. Agar (0.6 %) and sucrose (3 %) were added. This basal nutrient medium was modified by the addition of 150 mM NaCl, 10 mM proline or combination of them. The basal medium was used as control.

The embryos were grown for 7 d in a growth chamber [temperature of 24 °C photoperiod 14 h, irradiance of 222 μmol $m^{-2}$ $s^{-1}$ (400-700 nm)]. After 7 d, plants were harvested and lengths and fresh masses of roots and shoots were recorded.

Chlorophyll (Chl) contents of the leaves were measured after acetone extraction by spectrophotometer *Shimadzu UV 1208* (Tokyo, Japan) according to Arnon (1949). Protein contents of the tissues were determined spectrophotometrically by method of Bradford (1976) after extraction in 0.05 M phosphate buffer (pH 6.5), and proline contents were measured spectrophotometrically by the method of Bates *et al.* (1973).

All the experiments were repeated three times for each treatment.

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NaCl (150 mM) caused decrease in the elongation of both roots and shoots of the seedlings derived from bean embryos during seven days (Fig. 1). A small decrease in the fresh mass of both roots and shoots were also recorded (Fig. 1). Protein and Chl contents were also decreased by the NaCl. NaCl decreased protein content in roots but not in stem and leaves. The decreasing effect of NaCl on elongation and fresh mass of seedlings was alleviated in the combination of NaCl with 10 mM proline (Figs. 1, 2). Especially root growth was increased about two folds by proline and NaCl combination comparing to NaCl medium. Protein and chlorophyll contents also showed similar changes: proline and NaCl combination increased their contents in comparing to salt treatment (Figs. 1, 2). Proline was found to increase protein content especially in leaves. Endogenous proline contents of the seedlings were also increased by the NaCl or proline treatments (Fig. 2). In the case of proline and NaCl combination, proline content was increased more than in alone NaCl or proline treatment.

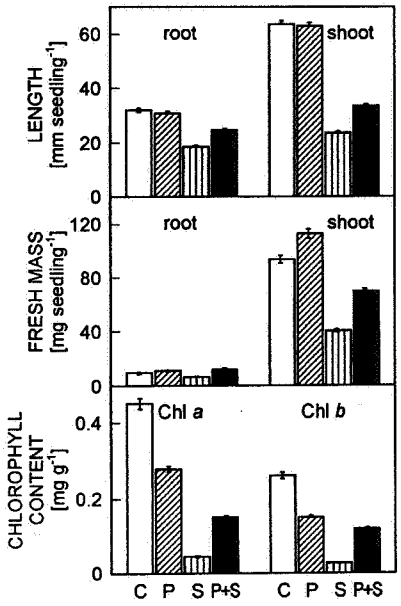


Fig. 1. Effect of 10 mM proline (P), 150 mM NaCl (S) and their combination (P+S) on length, fresh mass and chlorophyll *a+b* content of bean seedlings. Means ± SE for triplicate samples (C - control).

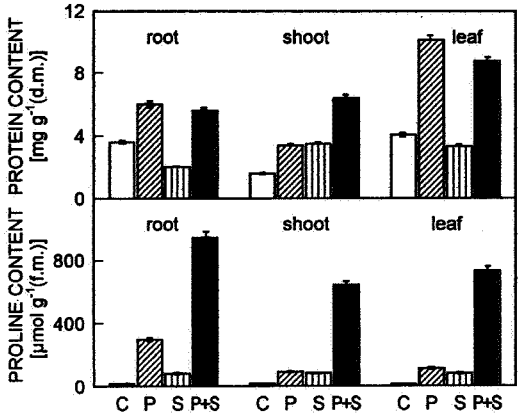


Fig. 2. Effect of 10 mM proline (P), 150 mM NaCl (S) and their combination (P+S) on contents of proteins and proline in bean seedlings. Means ± SE for triplicate samples (C - control).

Proline was seen to overcome salinity stress at 10 mM concentration. Lower concentrations (2.5 and 5.0 mM) of proline were also experienced in the study but they could not overcome salinity stress.

Decreasing protein content induced by salt has been reported previously (Hurkman *et al.* 1988), and proline addition has been found to increase protein content in saline medium (Bernard and Oaks 1969).

Proline accumulation has been indicated as tolerance criterion with related to the mechanism of osmoregulation in several plants (*e.g.* Stewart and Lee 1974, Wyn-Jones and Storey 1978). In our study also alleviation of salt stress by proline may be provided by this mechanism. Exogenously applied proline has been found to easily absorbed by roots and transported to stem and leaves (Pandey and Gonapathy 1985).

As conclusion, 150 mM of NaCl inhibited seedling growth but this inhibition was significantly reduced in the presence of 10 mM proline. This effect also reflected on chlorophyll and protein contents of the seedlings.

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