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BRIEF COMMUNICATION

Proline accumulation in two bean cultivars under salt stress and the effect of polyamines and ornithine

J.F. JIMÉNEZ-BREMONT*[1], A. BECERRA-FLORA*, E. HERNÁNDEZ-LUCERO*, M. RODRÍGUEZ-KESSLER*, J.A. ACOSTA-GALLEGOS** and J.G. RAMÍREZ-PIMENTEL***

*División de Biología Molecular, Instituto Potosino de Investigación Científica y Tecnológica, Camino a la Presa de San José 2055, 78210 Tangamanga, San Luis Potosí, SLP, México**
*Programa de Frijol, Campo Experimental Bajío-INIFAP, AP 312, 38000-Celaya, Guanajuato, México***
*Subdirección de Investigación y Graduados Agropecuarios, Instituto Tecnológico Agropecuario No. 33, K8 Carretera Celaya - Juventino Rosas, AP 508, 38110-Celaya, Guanajuato, México****

Abstract

Proline accumulation in two different bean (*Phaseolus vulgaris* L.) cultivars, one drought-sensitive (Canario 60) and one drought-resistant (Pinto Villa) was investigated. Both tolerated salt concentrations up to 150 mM NaCl, but the sensitive Canario 60 did not survive at 400 mM NaCl. In response to salt stress, both cvs. accumulated proline in all the analyzed tissues, the lowest contents were detected in roots. Pinto Villa accumulated higher proline concentrations than Canario 60 only at 400 mM NaCl. The addition of polyamines or ornithine increased proline content in plant tissues without stress, while they decreased it under salt stress.

Additional key words: *Phaseolus vulgaris*, putrescine, salt tolerance, spermidine, spermine.

Salinity is one of the most important abiotic stresses affecting common bean (*Phaseolus vulgaris* L.) development and productivity worldwide (Bayuelo-Jiménez *et al.* 2002, Wang *et al*. 2003). Inhibition of plant growth and even plant death by NaCl is due to a reduction in water availability, sodium ions accumulation and mineral imbalances. All of these factors manifest themselves by morphological, physiological and metabolic modifications in plants, such as decrease in seed germination, decrease in shoot and root length, alterations in the integrity of cell membranes, inhibition of different enzymatic activities and photosynthesis (Dash and Panda 2001, Sairam and Tyagi 2004). Complex molecular responses including the accumulation of compatible solutes, the production of stress proteins, and the expression of different sets of genes are part of the plant signalling and defence system against salinity (for reviews, see Hasegawa *et al.* 2000, Sairam and Tyagi 2004). It is well known, that one of the most common responses to water deficit and saline environments is the accumulation of proline (Pro) which acts as a compatible solute, an osmoprotectant, and a protective agent for cytosolic enzymes and cellular organelles (Delauney and Verma 1993, Bohnert *et al*. 1995, Taylor 1996, Demir and Oztürk 2003). Additionally, Pro is a nitrogen source available for recovery from stress and for restoration of growth (Trotel *et al*. 1996). Salt-induced Pro accumulation is often a late response, appearing only when cell injury is evident, and elevated levels of Pro are maintained long enough after stressed tissues return to normal osmotic conditions (Trotel *et al*. 1996).

Polyamines (PAs) have been involved in the regulation of many physiological processes in plants (rhizogenesis, somatic embryogenesis, pollen formation, flowering and initial fruitlet abscission, dormancy and senescence), and have been related to a number of

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Abbreviations: Orn - ornithine; PAs - polyamines; Pro - proline; Put - putrescine; Spd - spermidine; Spm - spermine.
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[1] Corresponding author; fax (+52) 444 8 342010, e-mail: jbremont@ipicyt.edu.mx

environmental challenges such as mineral nutrient deficiencies, osmotic stress, heat and chilling stress as well as salinity stress (for reviews, see Bouchereau *et al*. 1999, Kakkar *et al*. 2000, Sairam and Tyagi 2004). Changes in PAs contents may either induce Pro accumulation or control the Pro accumulation under stress conditions (Larher *et al*. 1998). The increased content of Pro or PAs biosynthesis in transgenic plants confers enhanced tolerance to abiotic stress (Kishor *et al*. 1995, Kasukawe *et al*. 2004).

In the present paper we investigated changes in Pro content under salt stress in two bean cultivars, one tolerant (Pinto Villa) and one sensitive (Canario 60) to drought stress (Acosta-Gallegos *et al.* 1995, Terán *et al*. 2002, Rosales-Serna *et al*. 2004 and Acosta-Gallegos, personal communication). The effect of exogenous administration of PAs, Pro and ornithine (Orn) (a common precursor of both, PAs and Pro) on this response was also investigated.

Phaseolus vulgaris L. seeds were germinated and grown in plastic pots (7 cm high and 10 cm diameter) containing 550 cm^3 of a sterile commercial horticultural grade soil mixture (*Cosmocel*, Monterrey, México) and irrigated with distilled water every 2 d. Plantlets were maintained in a growth chamber under controlled conditions [16-h photoperiod (150 µmol m^{-2} s^{-1}), 60 % relative air humidity, 25 ± 2 °C] for a period of 10 d after germination. Each experiment included twelve treatments with six replicates. At the seedling stage (10 d after germination) pots were supplied during 30 d with 3 cm^3 of each test solution as follows: the first group of plants received distilled water, the second, third and fourth groups received 25, 150 or 400 mM NaCl, respectively, the fifth group was irrigated with a PAs mixture [(1 mM putrescine (Put), 1 µM spermidine (Spd) and 1 µM spermine (Spm)], the sixth group received 5 mM Orn, the seventh group received 1 mM Pro, the eighth group received 150 mM NaCl and PAs mixture, the ninth group 150 mM NaCl and 5 mM Orn, the tenth group 150 mM NaCl and 1 mM Pro, the eleventh group PAs mix, 1 mM Pro and 5 mM Orn, and the last group, the same previous mixture plus 150 mM NaCl. At the end of the experimental period (30 d), all plants were harvested and frozen in liquid nitrogen until Pro determinations from leaves, stems and roots were done.

Free Pro was extracted by boiling 0.5 g of material in 2 cm^3 of distilled water. Then 0.5 cm^3 of sodium citrate (0.2 mM pH 4.6) and 2 cm^3 of 1 % ninhydrin (acetic acid-water, 60:40) were added to 0.5 cm^3 of the plant extract. Mixture was boiled 1 h, 2 cm^3 of toluene was added for the extraction and then centrifuged. Pro content was determined spectrophotometrically (*Beckman* Du-530, Fullerton, USA) at 520 nm following the ninhydrin method described by Magne and Larher (1992), using pure Pro (*Sigma-Aldrich*, Missouri, USA) as standard. All the experiments were done in triplicates and the data represent means ± SD.

Our results showed a noticeable difference among the examined cultivars. The drought-sensitive cv. Canario 60 did not survive at 400 mM NaCl, whereas, this salt concentration reduced growth of the drought-tolerant cv. Pinto Villa by 35 %. The Pro content of the two bean cultivars was enhanced at different salt concentrations (Fig. 1). The highest amount of Pro in leaves and stems was observed at 150 or 400 mM NaCl for the Canario 60 and Pinto Villa, respectively (Figs. 1*A*,*B*). Interestingly, Canario 60 accumulated three times more proline than Pinto Villa in stems at 150 mM NaCl (Fig. 1*B*). It is possible that the tolerant cultivar activates other mechanisms to counteract the salt effect, but at the highest NaCl concentration tested, the plant triggers Pro accumulation in order to survive. The concentrations of Pro in roots were lower than in leaves and stems in both cvs. (Fig. 1*C*). In the roots of Pinto Villa the highest Pro concentration was observed at 400 mM NaCl while in Canario 60 at 25 mM NaCl (Fig. 1*C*). According to our data, there must be a relationship between drought and salt resistance mechanisms in bean, since cv. Pinto Villa, originally isolated as a drought-tolerant cultivar, is also salt resistant.

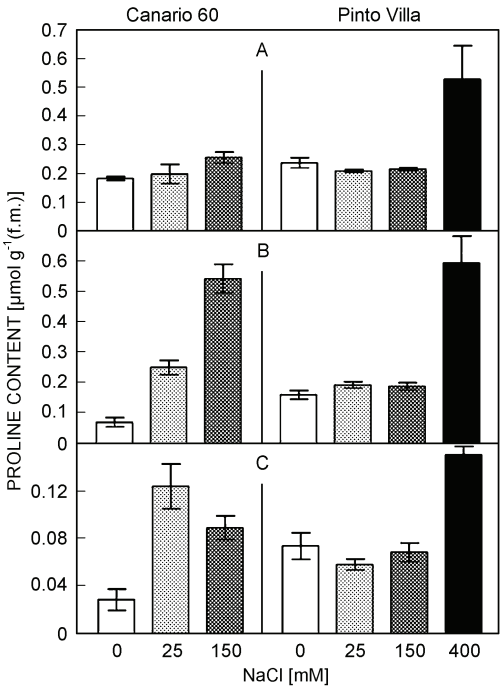

Fig. 1. Proline content in leaves (*A*), stems (*B*) and roots (*C*) of the two bean cultivars (Canario 60 and Pinto Villa) subjected to various concentrations of NaCl for 30 d. Means ± SD, $n = 6$.

A mixture of PAs (Put, Spd and Spm) also increased Pro concentrations in all the analyzed tissues of the sensitive and resistant cultivars, the effect on the former being higher (Fig. 2*A*-*C*). Bouchereau *et al*. (1999) demonstrated that exogenous Put supplied at low concentrations stimulates Pro accumulation. A similar

effect was observed in the present study. This observation suggests that polyamines trigger Pro accumulation, since the metabolic pathways involved in Pro and PAs biosynthesis require glutamate, ornithine and arginine as precursors; probably these pathways are finely coordinated. When sensitive plants were subjected to salt stress (150 mM) in the presence of PAs, the contents of Pro decreased in relation to the controls with PAs and NaCl alone; whereas such a decrease did not occur in resistant plants (Fig. 2). These data suggest that the salt

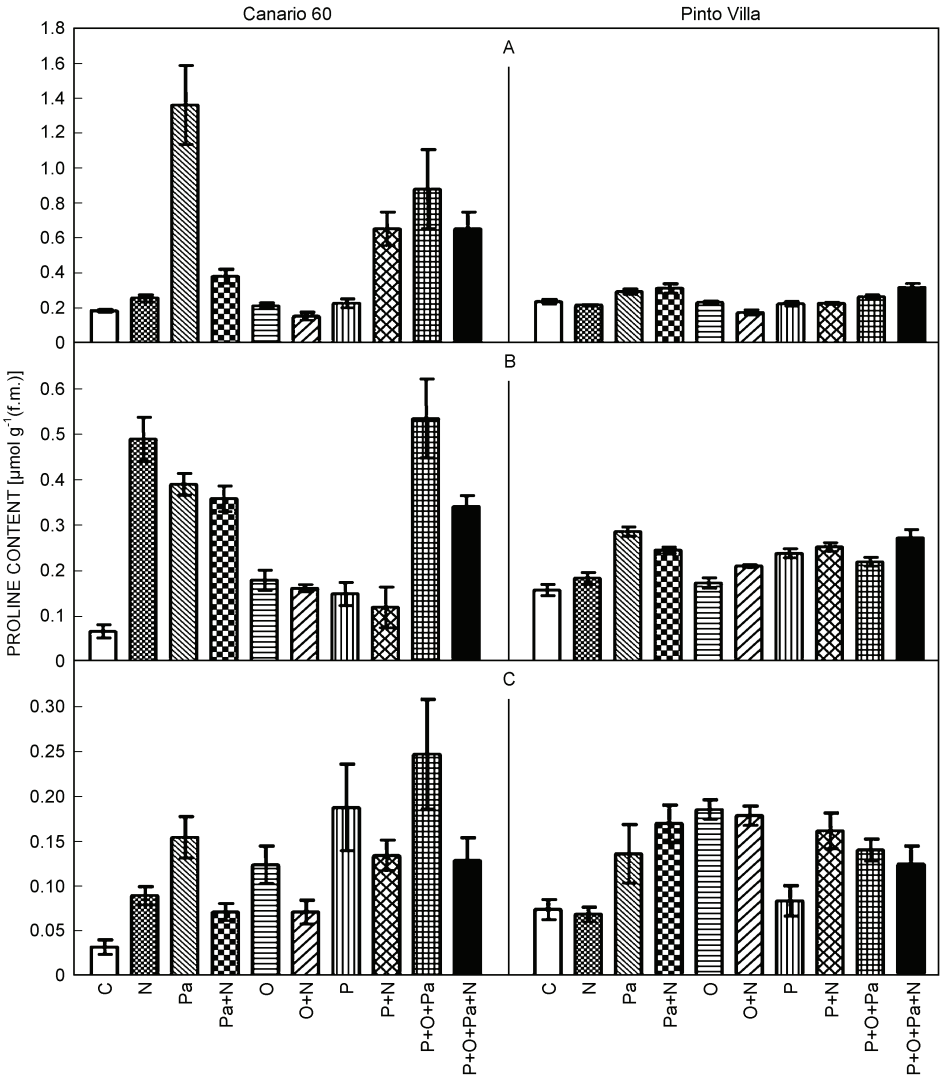

Fig. 2. Effects of exogenous polyamines, ornithine and proline on the salt-induced proline accumulation of two bean cultivars (Canario 60 and Pinto Villa). Proline content was measured in leaves (*A*), stems (*B*) and roots (*C*). C - untreated plants; 150 mM NaCl (N), mix of polyamines (1 mM Put, 1 µM Spd, 1 µM Spm, Pa), 5 mM ornithine (O) and 2 mM proline (P) and combinations of these solutions were added to the bean cultivars during 30 d. Means ± SD, $n = 6$.

stress may be partially alleviated by PAs, without requiring the accumulation of Pro to high levels. A similar response was observed upon the addition of Orn, although the increase of Pro accumulation was much lower than that observed after PAs addition. As a possible explanation we suggest that ornithine serves as a PAs precursor, thus relieving the necessity for Pro accumulation. In the presence of exogenous Pro, the content of the endogenous Pro increased in plant tissues only to significantly higher values in the roots, and the contents decreased upon salt addition, except in the leaves of Canario 60 and in the roots of the Pinto Villa, where they increased. Accordingly, it appears that Pro is not transported at a high rate from the roots to the leaves, but it is locally synthesized in response to different signals coming from the roots. The results obtained by

the addition of mixtures of PAs, Orn and Pro, suggest that their action is not additive regarding to Pro accumulation. It must be noticed that although the data obtained with the different additions to the resistant Pinto Villa were qualitatively similar to those obtained with the sensitive Canario 60, Pro accumulation was much lower, suggesting that the resistance of this cultivar to salt stress at this NaCl concentrations, probably depends on other mechanisms unrelated to Pro or PAs accumulation.

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