Cultivos Tropicales, 2015, vol. 36, no. 3, pp. 71-74
DOI: 10.13140/RG.2.2.24818.09923
http://dx.doi.org/10.13140/RG.2.2.24818.09923
july-september

ISSN impreso: 0258-5936
ISSN digital: 1819-4087
INCA
Ministerio de Educación Superior. Cuba
Instituto Nacional de Ciencias Agrícolas
http://ediciones.inca.edu.cu

EFFECT OF SALT STRESS ON SEED GERMINATION AND SEEDLINGS GROWTH OF *Phaseolus vulgaris* L.

Efecto del estrés salino en la germinación y el crecimiento temprano de *Phaseolus vulgaris* L.

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ABSTRACT. Common bean (*Phaseolus vulgaris* L.) is an important food legume worldwide. Salinity induces losses in common bean yield, particularly in arid, semiarid and in non-irrigated areas. The objective of this study was focused on determining effects of salt stress on seed germination and seedling growth of *Phaseolus vulgaris*. The common black bean cv. ICA Pijao was used as plant material. Salt stress was induced putting the seeds of this crop in different solutions of NaCl: 50, 100, 150, 200, 250 and 300 mM and deionized sterile water was used like control. The results showed that salinity stress reduced both seed germination and seedlings growth at salt concentrations above 150 mM. Consequently, results strengthen the role of NaCl as a stress-inducing factor, and also the susceptibility of common bean to salinity. Parameters evaluated in this work may provide new criteria to support *in vitro* selection in common bean breeding programs to find salt stress tolerant genotypes.

Key words: *in vitro*, abiotic stress, bean, growth, salinity

RESUMEN. El frijol común (*Phaseolus vulgaris* L.) es una leguminosa alimenticia importante a nivel mundial. La salinidad induce pérdidas en el rendimiento del frijol común, especialmente en las zonas áridas, semiáridas y en las zonas de secano. El objetivo de este estudio se centró en la determinación de los efectos de estrés salino sobre la germinación de semillas y el crecimiento de plantas de *Phaseolus vulgaris.* El frijol común (color negro) cv. ICA Pijao se utilizó como material vegetal. El estrés salino se indujo poniendo las semillas de este cultivo en diferentes soluciones de NaCl: 50, 100, 150, 200, 250 y 300 mM y se utilizó agua desionizada estéril como control. Los resultados mostraron que el estrés por salinidad redujo tanto la germinación de semillas como el crecimiento de las plántulas a concentraciones salinas por encima de 150 mM. En consecuencia, los resultados refuerzan el papel de NaCl como un factor inductor de estrés y también la susceptibilidad de frijol común a la salinidad. Los parámetros evaluados en este trabajo pueden proporcionar nuevos criterios para apoyar a la selección *in vitro* en los programas de mejoramiento de frijol común para encontrar genotipos tolerantes a estrés salino.

Palabras clave: *in vitro*, estrés abiótico, crecimiento, frijol, salinidad

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INTRODUCTION

Dry common bean (*Phaseolus vulgaris* L.) is consumed throughout the world. Bean is considered as the fourth source of protein in America, and it is especially important in the nutrition (1). In Cuba production of common beans in 2012 was 135, 000 tonnes.

Salt stress is an important constraint to world agricultural production. Salinity limits plant growth and productivity (2). It inhibits plant growth because affects important metabolic processes like photosynthesis, transpiration, cell growth and seed germination, this is the most perceptible phase in salinity conditions, because affect absorption of water (3).

A major problem of bean in Cuba is water stress caused by drought periods and salinity, resulting in losses of yield. Salinity-resistant cultivars have been developed in several breeding programs for crops like rice, sugarbeet, cotton, and barley. In general, germination and seedling growth parameters are the most viable criteria used for selecting salt tolerant crop plants; hence, percentage of germination and seedling growth are important growth parameters to be studied for cultivar selection (4). *In vitro* selection has emerged as a feasible tool for developing stress-tolerant plants. In order to be able to use *in vitro* selection, a better criteria and ecophysiological parameters associated with salt tolerance are needed for support bean breeding. The aim of this work was focused to determine *in vitro* effects of NaCl in *Phaseolus vulgaris* L. seed germination and seedling growth, under salt stress.

MATERIALS AND METHODS

The experiments were carried out under *in vitro* controlled condition (natural light and 28 ± 2 °C). Mature seeds of *Phaseolus vulgaris* (black bean) cv. ICA Pijao were surface disinfected with 70 % ($^v/_v$) ethanol for two minutes, followed by ten minutes in 3 % sodium hypochlorite ($^v/_v$) and rinsed with sterile water for three times. They were placed in sterile water for 24 hours, until complete imbibition occurred.

NaCl was used as selective agent to induce salt stress. Seven treatments were used, each with 10 repetitions: 0, 50, 100, 150, 200, 250 and 300 mM equivalent to 0, -0.2, -0.4, -0.6, -0.8, -1.0 and -1.2 MPa respectively (5). One imbibed seeds of *Phaseolus vulgaris* cv. ICA Pijao were placed in test tubes (2 cm of wide and 15 cm of length) on a support, this structure avoid a direct contact of solution with the seeds. Each tube contained 10 ml of NaCl solution or deionized sterile water as control.

Germination percentage (GP) was evaluated at 48 hours, according to following equation, where GS is number of germinated seeds (when the radicula emerged around 2 mm) at the end of experiment and TS is number of total seeds:

$$GP = \frac{GS}{TS} * 100$$

Growths parameters of germinated plants were measured at three and six days after exposure of seeds to salt stress induced by NaCl. Variables associated with growth were: stem length (cm) and root length (cm). Leaf area (LA) (cm^2) was determined using the equation (6), where L (length of leaf) and A (width of leaf):

$$LA = 0{,}603 + 0{,}581\ (L * A)$$

All data were analyzed statistically by Statistic Package for Social Science (SPSS) version 19.00 for Windows. Experiments were set up in a completely randomized design and each treatment had ten replicates, and they were repeated three times. Significant differences among treatment means were calculated by the Kruskall-Wallis and Mann-Whitney test at a probability level of 0,05.

RESULTS AND DISCUSSION

At 48 hours, seed germination in control is lower because were used a little number of replica. Germination was significantly reduced at 200 and 250 mM with regard to control (Table). NaCl presence stopped water absorption by the seeds of *Phaseolus vulgaris* cv. ICA Pijao.

In correspondence with this, was evaluated the effect of five different levels of salt (NaCl) on the germination of *Phaseolus vulgaris* L. seed and observed that an increase in salinity induces a reduction in the percentage of germinating and a delay in the initiation of the germination process (7). Furthermore, other authors observed seed germination inhibition in *Vicia faba* L. using NaCl at highest levels -0,9 and -1,2 MPa (8). In many cases the plant response under salinity conditions depends on cultivar, and this support genetical variability between them (9). *In vitro* salt stress induction using NaCl

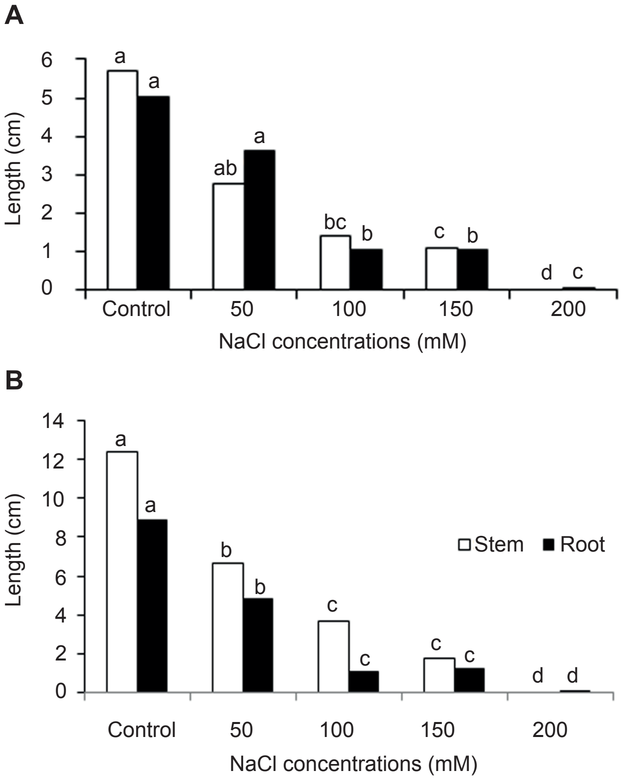
Table. Salt stress on seed germination of *Phaseolus vulgaris* cv. ICA Pijao at 48 hours (n=10 plants per treatment)

Na [Cl] (mM)	ΨH (MPa)	Germination (%)	Mean rank
Control	0	90	90,5 a
50	- 0,2	90	90,5 a
100	- 0,4	100	97,5 a
150	- 0,6	100	97,5 a
200	- 0,8	40	55,5 b
250	- 1,0	10	34,5 c
300	- 1,2	0	27,5 c

Values within a column followed by different lowercase letters are significantly different at p<0,05, according to Kruskal-Wallis and Mann Whitney test.
ΨH: Potencial hídrico

of *Phaseolus vulgaris* seeds will facilitate the evaluation of physiological parameters associated with germination that could be used to discriminate salinity tolerance in *Phaseolus vulgaris* cultivars in future experiments.

The growth (stem and root length) of *Phaseolus vulgaris* cv. ICA Pijao after three (Figure 1A) and six (Figure 1B) days was lower in plants exposed to NaCl solution in comparison with plants exposed to sterile water. Growth of plants at 200, 250 and 300 mM NaCl were drastically affected after three and six days with regard to control. Seeds that did not germinate produce no plants, means missing value. At six days, roots with the apical end stunted and dark brown coloration was observed in plants under salt stress.



ICA Pijao after: A) three days, B) six days. For each variable, different letters above columns indicate significant differences among treatments at P<0,05, according to Kruskal Wallis and Mann Whitney tests.

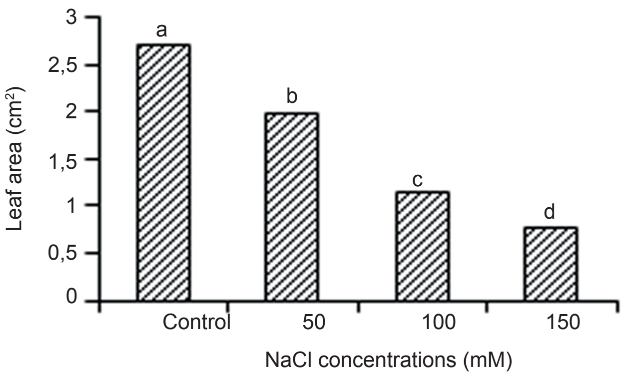
Figure 1. Effects of different NaCl concentrations on stem length and root length of *Phaseolus vulgaris* cv.

Similar results have been obtained by others authors, where vegetative growth of some crops is affected by salinity (10, 11). In previous research the root length in all cultivars (cv. Lody, cv. Gina and cv. Tara) of *Phaseolus vulgaris* was reduced as a result of the salt stress. The relative growth rates in the three cultivars were almost similar under non-saline conditions but declined considerably after salt treatment with 100 mM of NaCl (12). According to this, salinity at 200 mM NaCl reduced growth of stems and dry biomass of some *Vigna unguiculata* L. cultivars (13).

In this experiment, roots tend to be more branched. Primary roots were short with atrophic apical edges. However secondary roots were more thin and visible since the concentration of NaCl increase until 200 mM. In this sense, other researchers observed that salinity also produces a reduction in the stele diameter of root, because the decrease in number and diameter of the xylem vessels (14). Salt treatment decreased the percentage of pectin, but increased cellulose across root zones of two soybean cultivars. Suggesting that salt presence may increase cell wall rigidity, and thus, inhibiting root growth (15).

High salinity produces in plant a physiological drought. According to this, common bean duplicated the number of secondary and tertiary roots with modified architecture in response to drought (6). A deep and extensive root system has been advocated to increase productivity of food legumes under drought and salinity conditions. Root length could be an important trait to assist *in vitro* selection of salinity resistant varieties of beans with an improved capacity to acquire water.

Leaf area of *Phaseolus vulgaris* cv. ICA Pijao after six days was significantly reduced by salt stress in comparison with plants grown in sterile water (Figure 2). Furthermore, leaflets were wrinkled and with a thicker texture compared to the control. With higher concentrations of NaCl appeared necrotic spots in leaf edges.



Different letters above columns indicate significant differences between treatments at P<0,05, according to Kruskal-Wallis and Mann Whitney

Figure 2 Effects of different NaCl concentrations on leaf area of *Phaseolus vulgaris* cv. ICA Pijao after six days

Other authors inform that high concentrations of NaCl (120 and 240 mM) decreased the number of leaves or leaf area in *Vicia faba*. In this case, decrease in leaf area could be explained by the negative effect of salt on photosynthesis that leads to the reduction of plant growth, leaf growth, and chlorophyll content (16). The lower leaf areas per plant since concentration of NaCl increase indicate adaptive response of *P. vulgaris* plants for controlling water losses under salinity conditions. This factor may provide a selection criterion to find salt stress resistance genotypes in bean breeding program.

CONCLUSIONS AND RECOMENDATIONS

The present study confirms that *Phaseolus vulgaris* is susceptible to high concentrations of NaCl. The results showed that salinity stress reduced both seed germination and seedlings growth at salt concentrations above 150 mM. This variables may be used to find salt stress resistance genotypes *in vitro* culture, but more parameters like water content, fresh and dry weight, photosynthesis activity, phytotoxicity, should be studied for have a better criteria in the plant selection.

ACKNOWLEDGEMENTS

The project was jointly supported by Breeding National Program of *Phaseolus vulgaris* L. in Plant Biotechnology Institute.

BIBLIOGRAPHY

1. Romero, A.O.; Damián, H.M.A.; Rivera, T.J.A.; Báez, S.A.; Huerta, L.M. y Cabrera, H.E. "The nutritional value of beans (*Phaseolus vulgaris* L.) and its importance for feeding of rural communities in Puebla-Mexico", *International Research Journal of Biological Sciences*, vol. 2, no. 8, 2013, pp. 59–65, ISSN 2278-3202.
2. Khan, M.H. y Panda, S.K. ''Alterations in root lipid peroxidation and antioxidative responses in two rice cultivars under NaCl-salinity stress'', *Acta Physiologiae Plantarum*, vol. 30, no. 1, 2007, pp. 81-89, ISSN 1861-1664, DOI 10.1007/s11738-007-0093-7.
3. Meloni, D.A.; Gulotta, M.R.; Martínez, C.A. y Oliva, M.A. ''The effects of salt stress on growth, nitrate reduction and proline and glycinebetaine accumulation in Prosopis alba'', *Brazilian Journal of Plant Physiology*, vol. 16, no. 1, 2004, pp. 39–46, ISSN 1677-0420.
4. Mordi, P. y Zavareh, M. "Effects of salinity on germination and early seedling growth of chickpea (*Cicer arietinum* L.) cultivars'', *International Journal of Farming and Allied Sciences*, vol. 2, no. 3, 2013, pp. 70-74, ISSN 2322-4134.
5. Sosa, L.; Llanes, A.; Reinoso, H.; Reginato, M. y Luna, V. "Osmotic and specific ion effects on the germination of Prosopis strombulifera", *Annals of Botany*, vol. 96, no. 2, 2005, pp. 261–267, ISSN 1095-8290, DOI 10.1093/aob/mci173.
6. Barrios, A.N.; Ritchie, J. y Smucker, A.J. "El efecto de sequía en el crecimiento, la fotosíntesis y la intercepción de luz en frijol común", *Agronomía Mesoamericana*, vol. 9, no. 2, 1998, pp. 01–08, ISSN 1659-1321.
7. Cokkizgin, A. ''Salinity stress in common bean (*Phaseolus vulgaris* L.) seed germination'', *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, vol. 40, no. 1, 2012, pp. 177–182, ISSN 1842-4309.
8. Abd El-Samad, H.M. y Shadadd, M.A.K. "The Response Strategy of Maize, Pea and Broad Bean Plants to Different Osmotic Potential Stress'', *Journal of Stress Physiology & Biochemistry*, vol. 9, no. 3, 2013, pp. 240-250, ISSN 1997-0838.
9. Asmare, H.A. y Ambo, E. ''Impact of Salinity on Tolerance, Vigor, and Seedling Relative Water Content of Haricot Bean (*Phaseolus Vulgaris* L.) Cultivars'', *Journal of Plant Sciences*, vol. 1, no. 3, 2013, pp. 22–27, ISSN 2331-0731, DOI 10.11648/j.jps.20130103.11.
10. Martínez, N.; López, C.V.; Basurto, M. y Pérez, R. ''Efectos por salinidad en el desarrollo vegetativo'', *Tecnociencia Chihuahua*, vol. 5, 2011, pp. 156–161, ISSN 1870-6606.
11. Younesi, O.; Baghbani, A. y Namdari, A. "The effects of Pseudomonas fluorescence and Rhizobium meliloti co-inoculation on nodulation and mineral nutrient contents in alfalfa (*Medicago sativa*) under salinity stress'', *International Journal of Agriculture and Crop Science*, vol. 5, no. 14, 2013, pp. 1500–1507, ISSN 2227-670X.
12. Kaymakanova, M. y Stoeva, N. "Physiological reaction of bean plants (*Phaseolus vulgaris* L.) to salt stress'', *General and Applied Plant Physiology*, vol. 34, no. 3–4, 2008, pp. 177-188, ISSN 1312-8221.
13. Mahamadou, T.; Champion, A.; Diouf, D. y Ourèye, M. ''NaCl Effects on in vitro germination and growth of some senegalese cowpea (*Vigna unguiculata* (L.) Walp.) cultivars'', *ISRN Biotechnology*, 2013, pp. 1-11, ISSN 2090-9403, DOI http://dx.doi.org/10.5402/2013/382417.
14. Garzón, P. y García, M. "Efecto del estrés por NaCl sobre la anatomía radical y foliar en dos genotipos de Frijol (*Vigna unguiculata* (L.) Walp.)'', *Bioagro*, vol. 23, no. 3, 2011, pp. 153–160, ISSN 1316-3361.
15. An, P.; Li, X.; Zheng, Y.; Matsuura, A.; Abe, J.; Eneji, A.E.; Tanimoto, E. y Inanaga, S. "Effects of NaCl on Root Growth and Cell Wall Composition of Two Soya bean Cultivars with Contrasting Salt Tolerance", *Journal of Agronomy and Crop Science*, vol. 200, no. 3, 1 de junio de 2014, pp. 212-218, ISSN 1439-037X, DOI 10.1111/jac.12060.
16. Abdul Qados, A.M.S. ''Effect of salt stress on plant growth and metabolism of bean plant *Vicia faba* (L.)'', *Journal of the Saudi Society of Agricultural Sciences*, vol. 10, no. 1, enero de 2011, pp. 7-15, ISSN 1658-077X, DOI 10.1016/j.jssas.2010.06.002.

Received: September 23td, 2014
Accepted: January 27th, 2015