Available online at www.notulaebotanicae.ro

Print ISSN 0255-965X; Electronic 1842-4309

Not Bot Horti Agrobo, 2012, 40(1):177-182

Notulae Botanicae Horti Agrobotanici Cluj-Napoca

Salinity Stress in Common Bean (*Phaseolus vulgaris* L.) Seed Germination

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Abstract

In this study, the effect of five different levels of salt (NaCl) on the germination of *Phaseolus vulgaris* L. seed was investigated. Laboratory experiment with completely randomized design comprising three replicates in Petri dishes was conducted at Gaziantep University Vocational School of Higher Education in Nurdagi to determine the salt effects on common bean germination. The germination of the cultivar (Tegmen) was studied using distilled water (control) and under osmotic potential of 0.3, 0.6, 0.9, 1.2, and 1.5 MPa NaCl. The results indicated that the Mean germination time (MGT), Germination index (GI), Coefficient of velocity of germination (CVG), Germination percentage (GP), and Seed vigor index (SVI) varied between 3.13 and 3.78 days, 6.88 and 3.93, 0.156 and 0.153, 90 and 60%, and 867.0 and 290.3, respectively. Significant differences were found among NaCl treatments in terms of GI ($p<0.05$), GP ($p<0.01$), and SVI ($p<0.01$). All the examined parameters were decreased with increasing NaCl concentration, except MGT. The max and min GI, GP, CVG, and SVI were observed at the control condition (0.0 MPa) and highest osmotic potential (1.5 MPa) of NaCl, respectively. Correlation coefficient between all possible combinations was estimated and the results indicated that MGT, GI, GP, CVG, and SVI had significant positive or negative correlation with each other.

Keywords: common bean (*Phaseolus vulgaris* L.), correlation, germination, salinity stress, sodium chloride (NaCl)

Introduction

The genus *Phaseolus* L. includes numerous wild and cultivated species, originating from New World. Although the exact number is still unknown (Debouck, 1999), this genus has approximately 50 species (Delgado, 1985; Lackey, 1983). Two major gene pools of common bean were first recognized in the wild form, Mesoamerican and Andean (Gepts, 1998).

Phaseolus is cultivated on all continents except Antarctica, under very diverse cultivation conditions. Among the Asian countries, China, Iran, Japan, and Turkey are the major producers of the common bean (Singh, 1999).

Among the pulse crops (i.e. annual leguminous food crops), the common bean is by far the most important (Singh, 1999).

Salinity reduces the ability of plants to utilize water and causes a reduction in growth rate, as well as changes in plant metabolic processes (Munns, 1993, 2002). Furthermore, it decreases plant growth and yield, depending on the plant species, salinity levels, and ionic composition of the salts (Yadav *et al.,* 2010).

Saline stress is one of the main factors limiting legume productivity in arid and semi-arid regions (Lluch *et al.,* 2007), and salinity has direct harmful effects on numerous plant species (Cordovilla *et al.*, 1994; Greenway and Munns, 1980; Keck *et al.*, 1984). Salinity adversely affects the plant growth and development, hindering seed germination (Dash and Panda, 2001). Seed germination is usually the most critical stage in seedling establishment, determining successful crop production (Almansourie *et al.*, 2001; Bhattacharjee, 2008).

The germination of seed is a complex process depending on the genetic and environmental factors, such as temperature, light, and salinity (Barbour, 1968; Mahmoud, 1985).

Salinity is one of the most important factors limiting plant growth and delaying seed germination as well as final germination percentage (Rahman *et al.*, 2000).

The effect of salinity on plant growth is related to the stage of plant development at which salinity is imposed (Chartzoulakis and Loupassaki, 1997). Salinity stress affects seed germination either through osmotic effects, by preventing or delaying germination (Welbaum *et al.*, 1990), or through ion toxicity, which can render the seeds unviable (Huang and Reddman, 1995). However, Bayuelos *et al.* (2002) reported that increase in salinity from 0 to 180 mM of NaCl decreases germination by 50% in species of the genus *Phaseolus.*

In this study, the effects of salinization on the germination of bean were examined through laboratory experiment. The aim of this study was to assess the effects of sodium chloride (NaCl) on the germination of common bean seeds.

Materials and methods

Seed material

This study was conducted under the controlled environmental conditions at Gaziantep University Vocational

School of Higher Education in Nurdagi during November and December 2011.

A bean cultivar Tegmen was used in the laboratory research. It is a nationally registered dwarf bean cultivar, suitable for open-field production, and 1000 seed weight is 189 g.

NaCl Solutions and seed treatments

Bean seeds were surface-sterilized with 5% sodium hypochlorite (NaOCl) (Sauer and Burroughs, 1986) and washed thoroughly with distilled water (2.5 µs/cm).

The seeds were then germinated in 120-mm-diameter sterilized Petri dishes. All Petri dishes were washed with tap water, followed by a rinsing with distilled water, and then sterilized at 170°C for 4 h in hot air sterilizer (Muhammad and Hussain, 2010).

NaCl solutions were prepared with osmotic potentials of 0.0 (Control), -0.3, -0.6, -0.9, -1.2, and -1.5 MPa (0.0, 3.5, 7.1, 10.6, 14.2, and 17.7 g/liter NaCl) (Goertz and Coons, 1991).

The Petri dishes were arranged in a completely randomized block design with three replications. A total of 20 seeds were put in each Petri dish on double-layer Whatman paper, and 10 ml of appropriate solution was added to each Petri dish (Asgharipour and Rafiei, 2011).

Subsequently, the seeds were imbibed in NaCl solutions for 24 h at 20 ± 0.1°C temperature. The seeds were then drained, rinsed twice with distilled water, and were allowed to continue germination on moist double-layer new Whatman paper in the dark (Asgharipour and Rafiei, 2011).

The germination cabinet was sprayed with Captan (N-Trichloromethylthio-4-cyclohexene-1,2-dicarboximide) to prevent fungal growth. The Petri dishes were placed in the germination cabinet at a temperature of 20 ± 0.1°C and 50 ± 0.5% relative humidity (RH) for 9 days (ISTA, 2011).

During this period, the Petri dishes were observed daily. Each day, 5 ml of distilled water (2.5 µs/cm) was added to the Petri dishes.

Mean germination time

The seedlings' stunted primary roots were considered as abnormally germinated (ISTA, 2011). A bean seed was considered to have germinated when the radicle reached a length of 10 mm (Goertz and Coons, 1989).

The mean germination time (MGT) was calculated to assess the rate of germination (Ellis and Roberts, 1981) as follows:

$$\mathrm{MGT} = \frac{\sum(\mathrm{Dn})}{\sum \mathrm{n}}$$

where *n* is the number of seeds germinated on each day and *D* is the day of counting. Cotyledons were not included in fresh and dry weight comparisons.

Germination index

Germination Index (GI) was calculated as described by the Association of Official Seed Analysts (AOSA, 1983) as:

$$\mathrm{GI} = \sum\left(\frac{\mathrm{GT}}{\mathrm{Tt}}\right)$$

or

$$\mathrm{GI} = \left[\frac{\text{Number of germinated seeds in first count}}{\text{Days of first count}}\right] + \ldots + \left[\frac{\text{Number of germinated seeds in final count}}{\text{Days of final count}}\right]$$

Coefficient of velocity of germination

Coefficient of velocity of germination (CVG) was evaluated according to Maguire (1962) as follows:

$$CVG = \frac{(G_1 + G_2 + \ldots + G_n)}{(1xG_1 + 2xG_2 + \ldots + nxG_n)}$$

where *G* is the number of germinated seeds and *n* is the last day of germination.

Germination percentage

The germinated seeds were counted daily according to the seedling evaluation procedure described in the Handbook of Association of Official Seed Analysts. The number of germinated seeds was recorded every 24 h (AOSA, 1990). Ten days after germination, the germination percentage (GP) was obtained by dividing the number of germinated seeds in any Petri dishes by the total number of seeds, multiplied by 100 (Cokkizgin and Cokkizgin, 2010; Tanveer *et al*., 2010)

Seed vigor index

Seed vigor index (SVI) was calculated according to Baki and Anderson (1973) as follows:

$$\mathrm{SVI} = [\text{Seedling length (cm) x GP (\%)}]$$

Statistical analysis

The experimental design comprised complete randomized blocks (CRD) with three replicates. The results were evaluated by analysis of variance using the Statistical Analysis System software v.9.0 (SAS, 2004), and treatments means were considered significantly different at $p<0.05$. Mean separation was evaluated by Least Significant Difference (LSD) test (Duzgunes *et al*., 1983).

Results and discussion

Mean germination time

No significant differences were observed among the NaCl treatments in terms of MGT (Tab. 1). In this study, all the examined parameters decreased with increasing NaCl concentration, except MGT (Tab. 2). The highest MGT was observed at 1.5 MPa (3.78 days) NaCl osmotic potentials, and the lowest was observed at 0.0 MPa (3.13 days). The present results agree with those reported by Pujol *et al*. (2000), who observed that an increase in salinity induces both a reduction in the percentage of germinating

Tab. 1. Summary of the analysis of variance for all the analyzed parameters

Source of Variation	d.f.	Means of Square				
		MGT	GI*	CVG	GP**	SVI**
NaCl Osmotic Potentials	5	0.1497	3.6530	0.00000462	465.56	143139.4
Error	12	0.4262	1.0180	0.00002061	43.06	5862.2
Variation coefficient %		18.57	19.43	2.94	9.30	13.51

*,** Significant at 0.05 and 0.01 probability levels, respectively

Tab. 2. Salinity effects for all parameters on germination in common bean

NaCl Osmotic Potentials	MGT (days)	GI	CVG	GP (%)	SVI
0.0 (Control)	3.13 a	6.88 a	0.156 a	0.90 a	867.0 a
0.3 MPa	3.44 a	6.14 ab	0.156 a	0.82 a	750.0 ab
0.6 MPa	3.48 a	4.90 bc	0.154 a	0.68 b	622.4 b
0.9 MPa	3.67 a	4.87 bc	0.154 a	0.62 b	451.9 c
1.2 MPa	3.60 a	4.43 bc	0.153 a	0.62 b	418.2 dc
1.5 MPa	3.78 a	3.93 c	0.153 a	0.60 b	290.3 d
LSD value	1.1613	1.795	0.0081	11.673	136.21

Means followed by the same letter are not significantly different at $p<0.05$ level (LSD test)

seeds and a delay in the initiation of the germination process. It can also cause a complete inhibition of the germination process.

Correlation coefficient between all possible combinations was estimated and is shown in Tab. 3. The results indicate that MGT has a highly significant negative correlation with GI (-0.926**), CVG (-0.823*), GP (-0.931**), and SVI (-0.950**).

NaCl concentrations were beyond the tolerance limits of the species. It has been reported that salinity delays germination (Ayers and Westcot, 1985; Mensah and Ihenyen, 2009; Murillo-Amador *et al.*, 2002; Rahman *et al.*, 2000; Welbaum *et al.*, 1990). Fig. 1A shows the relationship between the MGT and NaCl osmotic potentials of common bean seeds. It can be observed that the common bean seeds are affected negatively under salinity stress conditions. At high NaCl osmotic potentials during germination, salinity deteriorated seed-germination features.

Germination index

Significant differences were found among NaCl treatments in terms of GI ($p<0.05$). GI increased when the NaCl osmotic potential decreased. The highest GI, i.e. 90%, was observed in the control. Fig. 1B shows the change in GI with different NaCl osmotic potentials, which is similar to the results reported by Kaya *et al.* (2008).

GI exhibited positive and significant correlation with CVG (0.966**), GP (0.975**), and SVI (0.975**), and negative significant correlation with MGT (-0.926**).

Coefficient of velocity of germination

Nonsignificant differences were observed among NaCl treatments with respect to CVG. However, considerable decrease was noted in CVG, depending on NaCl osmotic potential. Fig. 1C shows the CVG at different NaCl osmotic potentials. These results are also in agreement with those reported by Katembe *et al.* (1998).

On the other hand, CVG showed positive and significant correlation with GI (0.966**), GP (0.950**), and SVI (0.933**), but negative and significant correlation with MGT (-0.823*).

Germination percentage

GP was significantly affected by NaCl osmotic potential (Tab. 3). Maximum GP (90%) was recorded in the control (0.0 MPa NaCl), while the lowest GP (60%) was recorded for 1.5 MPa NaCl osmotic potential. Fig. 1D shows the change in GP with different NaCl osmotic potentials. Furthermore, GP exhibited a positive significant correlation with GI (0.975**), CVG (0.950**), and SVI (0.955**), and a strong negative association with MGT (-0.931**).

Tab. 3. Correlation matrix for analyzed variables

	MGT	GI	CVG	GP	SVI
MGT	1.000				
GI	–0.926**	1.000			
CVG	–0.823*	0.966**	1.000		
GP	–0.931**	0.975**	0.950**	1.000	
SVI	–0.950**	0.975**	0.933**	0.955**	1.000

*,** Significant at 0.05 and 0.01 probability levels, respectively

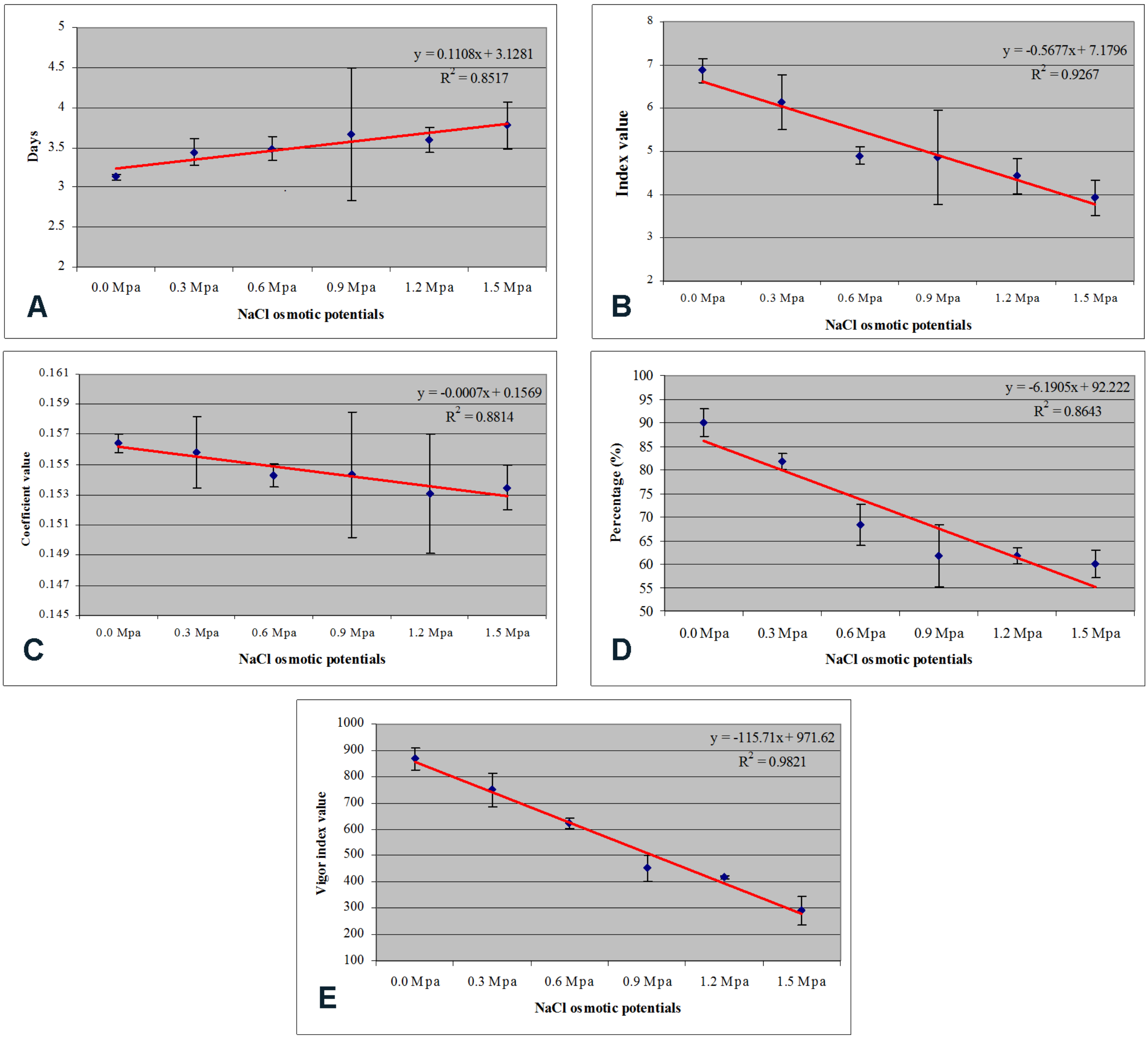

Fig. 1. Relationship between MGT(A), GI(B), CVG(C), GP(D), SVI(E), and NaCl osmotic potentials in common bean (*Phaseolus vulgaris* L.) seeds

The GP decreased when the NaCl osmotic potential increased. Furthermore, NaCl stress had a negative impact on GP (Kaymakanova, 2009). The final GP of the seeds pretreated with high salt concentrations was much lower than that of the control seeds, indicating that exposure to high concentration of NaCl strongly affected germination (Bayuelos *et al.*, 2002; Coons *et al.*, 1990; Duros and Magne 2008; Eroglu, 2007; Ungar, 1996). Similarly, Katembe *et al.* (1998) also reported that imbibitions of water following NaCl treatments decreased with the decrease in water potential of the solutions. Increasing concentration of NaCl is probably caused by the decrease in water potential gradient between the seeds and their surrounding media (Bradford, 1995). Shokohifard *et al.* (1989) pointed out that salinity stress negatively affects seed germination, either osmotically through reduced water absorption or ionically through the accumulation of Na^+ and Cl^-, causing an imbalance in the nutrient uptake and toxicity effect. According to the correlation coefficient, GP was found to have a positive and significant correlation with other measured parameters.

Seed vigor index

Significant differences were observed between NaCl treatments and SVI. Furthermore, considerable decrease in SVI was observed, depending on the increase in NaCl osmotic potential. The highest SVI was observed in the control (867.0), whereas the lowest was noted at 1.5 MPa NaCl osmotic potential (290.3). Fig. 1E shows the relationship between SVI and NaCl osmotic potentials.

The results indicate that all the parameters have highly significant correlation with SVI. The effects of GI (0.975**), CVG (0.933**), and GP (0.955**) were positive on the SVI, while that of MGT (-0.950**) on the SVI was negative.

The SVI increased when the NaCl concentration decreased, which shows that increased NaCl concentration caused a harmful effect in the seed. Similar results were observed by Khajeh-Hosseini *et al*. (2003). Ungar (1996) indicated that seedling length decreased significantly with increased salinity. Similarly, Al-Mutawa (2003) reported that increased salinity also leads to decreased radicle lengths. On the other hand, SVI had positive and significant correlation with GP, CVG, and SVI, except MGT.

Conclusions

In conclusion, this study demonstrated that bean seed germination varied according to the change in NaCl osmotic potential, i.e. NaCl has direct harmful effects on common bean seed germination (*Phaseolus vulgaris* L). At the germination stage, common bean was found to be sensitive to high-level salt osmotic potentials. Furthermore, the correlation coefficient results indicate that all the possible combinations had a positive significant correlation with each other, except the MGT coefficients.

Acknowledgments

The author is grateful to Gaziantep University, Scientific Research Projects Management Unit, and Prof. Dr. Mustafa Colkesen and Instructor Hatice Cokkizgin for help their during this experiment.

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