EFFECT OF SALINITY ON GERMINATION AND SEED PHYSIOLOGY IN BEAN *(PHASEOLUS VULGARIS L.)*

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ABSTRACT

The response of three bean cultivars (Phaseolus vulgaris L.) to equimolar NaCl and Na_2SO_4 salinity at germination and early seedling growth was investigated. Seeds were germinated and grown in Petri plate on filter paper with solution of the respective treatment and incubated at 25◦C in a thermostat. Each treatment was replicated three times. The seedlings were harvested and their growth, as well as their germination and respiration rates were measured.

All treated cultivars registered decrease in the percentage of germination, seedlings growth and respiration rate. The cultivars were inhibited stronger by Na_2SO_4 than NaCl treatment.

Keywords: bean, germination, growth, respiration, salinity

Introduction

During their growth crop plants are usually exposed to different environmental stresses which limit their growth and productivity. Among these, salinity and drought are the most severe ones. It has been estimated that more than 20% of all cultivated lands around the world contain salt levels high enough to cause salt stress to crop plants (10).

In saline environment adaptation of plants to salinity during germination and early seedling stages is crucial for the establishment of species. Seedlings are the most vulnerable stage in the life cycle of plants and germination determines when and where seedling growth begins (7). There are contradictory reports in the literature as to the relative sensitivity of germination and seedling growth to salt stress. According to Munns (11), salt stress decreases growth in most plants, including halophytes.

Salt stress affects many physiological aspects of plant growth. Shoot growth and dry matter are reduced by salinity, root: shoot ratio is increased (13). Miquel et al., (9) documented, that respiration decreases under water stress condition, though decrease in respiration is much less than photosynthesis. It was also proven that salt stress increases the activity of an alterative pathway along with the cytochrome pathway. Salinity can affect germination of seeds either by creating osmotic potential which prevent water uptake, or by toxic effects of ions on embryo viability (7). Shoot growth was reduced by salinity due to the inhibitory effect of salt on cell division and enlargement in the growing point (8). Nevertheless, the relative importance of osmotic and ionic effects on early growth of halophytes is still incomplete and depends on the species under study. Many studies on seedling growth response to salt stress have used seedlings pre-germinated under non-saline conditions. This approach may provide a clear separation of effects, but could give an unrealistic view of the response of seeds to saline types (2). Improving salt tolerant varieties is of major importance and efforts should be focused on finding mechanisms which are involved in salinity tolerance (10). This may induce us to find methods for screening a large number of genotypes for salt tolerance. Studies with seeds and early seedlings are carried out mostly with Triticum aestivum L. (13) and Prosopis strombulifera (7). The majority of salt stress studies have used NaCl as experimental salt. Few research works have recently reported results on the effects of Na_2SO_4 on germination and plant growth.

The aim of the present study was to evaluate the effect of monosaline iso-molar solutions of NaCl and Na_2SO_4 salinity at germination and early seedling growth stage.

Materials and methods

Bean seed (Phaseolus vulgaris L.) cultivars: Lody, Gina and Tara were surface sterilized with $2500 mgL^{-1}$ sodium hypochlorite solution for 5 min, rinsed with sterile distilled water several times, and briefly blotted onto sterile paper towels.

The experimental design with the three treatments was arranged for every cultivar:

1. Control – seeds germinated in deionized water;
2. Seeds, germinated in a solution of 100mM NaCl;
3. Seeds, germinated in a solution of 100mM Na_2SO_4.

The experiment was conducted in Petri plate on filter paper beds in a thermostat. 20 seeds were sown in Petri plate and germinated on papers imbibed in distilled water or in sodium chloride solutions or sodium sulphate solutions at a proportion of 2.5 times the weight of the paper. The Petri plate incubated at 25°C. The filter paper beds were changed after 48 hours in order to avoid salt accumulation (13).

Water uptake: Water uptake was recorded for 12 hours and the per cent was calculated under the following formula (13):

Water uptake, % = $[(W_2-W_1)]/W_1]x100$; where W_1 was the initial weight of seeds and W_2 was the weight of seeds after water absorption.

Germination: The emergence of radical/plumule or root/shoot from seeds was taken as an index of germination (%) which was recorded daily for up to 9 days.

After that salt tolerance was calculated using the following ratio (13):

Salt tolerance = A/B x 100; where

A = germination/growth in treated seedlings, and

B = germination/growth in control plants.

The seedling growth evaluation was carried out in Rules (12). Ten seeds were distributed on the germination paper, pre-imbibed in NaCl, Na_2SO_4 and distilled H_2O solutions. After 9 days in a 25°C germinator, the shoot length (SL), root length (RL), shoot weight (SW) and root weight (RW) were measured. The total seedling length (TL) and the RL:SL ratio were calculated out of these data.

The respiration was measured with a portable infrared gas analyzer *LCA-4 (Analytical Development Company Ltd., Hoddesdon, England),* equipped with a *PLCB-4* chamber in dark. Each treatment was replicated thrice.

Statistical analysis

Statistical analysis was performed using one-way ANOVA (for P<0.05). Based on the ANOVA results, a DUNCAN-test for mean comparison was performed, for a 95% confutation level, to test for significant differences among treatments. In the tables, different letters (a,b,c) after the values within the same column express significant difference.

Results

Germination percentage:

The results of seed germination are shown in **Figure 1**. Specific ionic effects were evident as different responses were obtained with different salt treatments at iso-molar concentration. It was observed that the germination of seeds treated with Na_2SO_4 is stronger inhibited than that treated with NaCl. This tendency was observed in the three cultivars, but it was most strongly expressed in Lody cultivar.

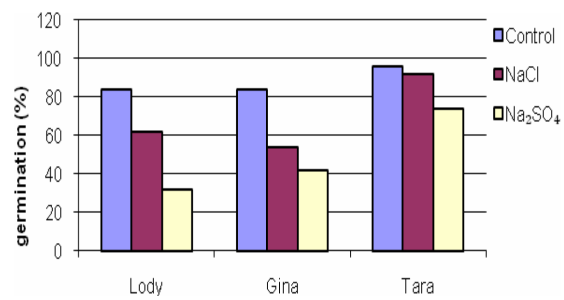

Fig.1. Total germination of seed of tree bean cultivars submitted to salt stress.

Seedling growth

The results from the initial growth of the seedlings and roots also show presence of considerable inhibition in the salt-treated variants (**Table 1**). It is seen that the inhibiting effect of Na_2SO_4 is by 20% stronger than that of NaCl. This tendency is observed in the three cultivars, but is strongest in Gina cultivar both with respect to the hypocotile growth and the root. Although both salts show inhibiting effect, that of Na_2SO_4 is stronger again and in Gina cultivar inhibition reaches up to 80% of the control. As regards to RL/HL and WR/WH ratio, the salt-treated seeds show results above the control. With respect to RL/HL they exceed it by approximately 30-40% and with respect to the second index (WR/WH) – by 60% above the control (Gina and Tara cultivars).

Water uptake and respiration

The results from **Figure 2** show that water uptake is reduced in salt-treated seeds, compared with those treated with distilled water. In this way when seeds of Lody cultivar are treated with NaCl, the inhibition of this index is 17% and in the variant treated with Na_2SO_4 – 32% of the control. The inhibition under this index is lower compared to both salts.

The data represented on **Figure 2** show too, that salinity stress has a negative effect on the dark respiration of the seeds. The results obtained for the three cultivars show a similar tendency.

TABLE 1.

Effect of salinity on early seedling growth LH-hypocotil length (cm); LR-root length (cm);TL –total length; WH-hypocotil biomass (g); WR root biomass (g).

Parameters	LH	LR	TL	RL/HL	WH	WR	WR/WH
Lody-control	5.92a	4.60a	10.58a	0.91	0.345a	0.117a	0.232
NaCL	3.75b	3.75a	7.38b	0.95	0.302b	0.082b	0.271
Na□SO□	2.47c	2.53b	5.03c	1.05	0.116c	0.045c	0.281
Gyna-contr	6.60a	2.80a	9.28a	0.48	0.787a	0.111a	0.141
NaCL	3.35b	2.20a	6.01b	0.85	0.375b	0.062b	0.165
Na□SO□	1.15c	1.05b	2.10c	0.76	0.153c	0.035c	0.228
Tara-contr	5.90a	2.40a	8.30a	0.52	0.435a	0.075a	0.172
NaCL	3.89b	2.08a	5.90b	0.55	0.232b	0.055b	0.237
Na□SO□	2.36c	1.63b	4.30c	0.68	0.132c	0.037c	0.280

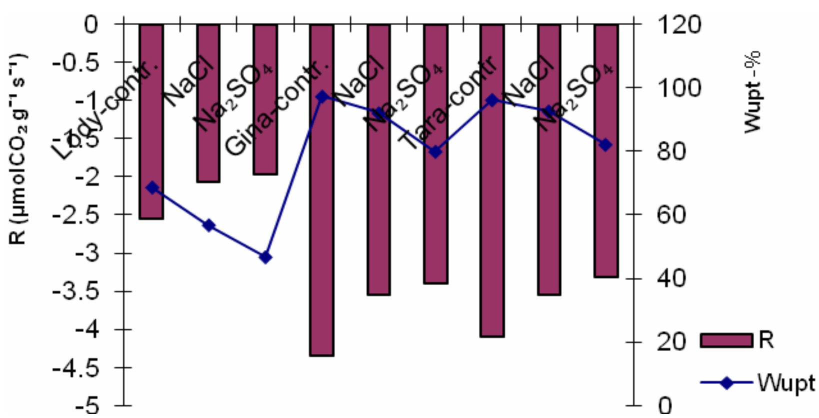

Fig.2. Effect of salinity on water uptake (Wupt-%) and dark respiration of seedlings [R – dark respiration (µmolCO_2 g^{-1} s^{-1})].

The process inhibition is more considerable in seeds treated with Na_2SO_4 compared to those treated with NaCl.

Salt tolerance

Data regarding salt tolerance of different cultivars under investigation (**Table 1**) show that cultivar Tara is most tolerant to NaCl and Na_2SO_4 salinity. On the basis of the tolerance to both salts the cultivars can be arranged as follows:

NaCl: Tara > Lody > Gina

Na_2SO_4: Tara > Gina > Lody

Discussion

Salinity affects germination in two ways:

There may be enough salt in the medium decrease the osmotic potential to such a point which retard or prevent the uptake of water necessary for mobilization of nutrient required for germination (**Fig.2**).

The salt constituents or ions may be toxic to the embryo.

Our results corresponded to these of Rahman et all, (2008), that germination was directly related to the amount of water absorbed and the delay in germination to the salt concentration of the medium. The salt tolerance of plants varies with the type of salt and osmotic potential of the medium (5).

Water availability is one of the main environmental factors limiting photosynthesis and growth (6). Salinity affects the seedling growth of plants by slow or less mobilization of reserve foods, suspending the cell division, enlarging and injuring hypocotyls (13). Nevertheless, there may not be a positive correlation between salt tolerance at germination stage and during later phases of growth. According to these authors the greater tolerance to salinity during germination is associated with lower respiration rates and greater reserve of respiratory substances.

Under salt stress conditions elongation rate of coleoptiles may decrease by low soil water potential (1) and seedlings may not be established well due to weak coleoptile and root growth. Reduced seedling growth has also been reported by Huang and Reddman (3) on barley and Jeannete et al., (4) on phaseolus under salt stress conditions. It can be concluded that to select cultivars for better salt stress tolerance at seedling stage, coleoptile and root elongation may be used as breeding criteria. Our results confirm the one of Moud, 2008 (10), that salt stress inhibits coleoptile growth more than root growth. Similar results were found by Jeanette et al., (4). This may in turn have the advantage of increased ratio of water absorption to transpiration area, a plant feature which is useful for dry land conditions if it lasts longer during other growth stages.

In the present study seedling respiration was decreased in accordance with the seedling growth as a salt stress type. According to some authors (10) correlation between seedling growth characteristics and respiration shows that under salt stress condition more respiration results in more vigorous seedlings, which may increase the degree of crop establishment. A comparison between the experimental data for the inhibition and growth shows that there is a correlation between them. Most significant is the inhibition of the two indices in Lody cultivar. More comprehensive studies are necessary for clarification of the differences between the different cultivars for maintenance of respiration during the period of salinity stress. The present study shows that bean seedling growth decreases in different type of salinity, due to the seedlings being unable to adjust osmotically or due to the toxic effects of Cl^-, SO_4^-, and/or Na^+. Probably, turgor of NaCl treated seeds maintained better water uptake into the tissues which together with the low concentration of tissue Na^+, compared with Na SO_4^- treated seeds, could be the cause of the smaller decrease in growth. In our opinion, it is possible effects upon water uptake to be the primary and predominant response of bean seedlings to salinity and further investigations are required in order to assess the physiological basis of this response.

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