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The Response of Some Haricot Bean (*Phaseolus vulgaris*) Varieties for Salt Stress during Germination and Seedling Stage

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Abstract: Fourteen haricot bean (*Phaseolus vulgaris*) varieties were tested during germination and seedling growth at 0, 2, 4, 8 and 16 dS/m salinity levels. Data analysis was carried out using jmp5 statistical software (version 5.0). Final Germination Percentage (FGP), Seedling Shoot Length (SSL), Seedling Root Length (SRL) and seedling Shoot-to-root Ratio (SRR) were measured. The data analysis showed insignificant variation among most parameters recorded for varieties ($p>0.05$). The ANOVA displayed statistical significance for treatments for all parameters at $p<0.0001$. However, it was insignificant for variety*treatment interaction ($p>0.05$). Seedling root length was more salt affected than seedling shoot length. Variety Awash Melka was found salt tolerant during germination and seedling growth. Variety Mexican 142 was salt sensitive during germination but later became salt tolerant during seedling growth. On the other hand, variety Dimtu was salt sensitive during germination and seedling growth. The rest haricot bean varieties were intermediate in their salt tolerance. The study affirmed the presence of broad intraspecific genetic variation in haricot bean varieties for salt tolerance. Irrespective of salinity being a growing problem in Ethiopia in general and the Awash Valley in particular, only little has been done on crops salt tolerance. Therefore, to alleviate the salinity problem, there should be similar and profound studies on haricot beans and other crops.

Key words: Germination, haricot bean, NaCl, salinity, seedling growth, varieties

INTRODUCTION

Salt-affected soils are distributed through out the world and no continent is free from the problem (Brady and Weil, 2002). Salinization of soil is one of the major factors limiting crop production particularly in arid and semi-arid regions of the world (Ahmed, 2009). Globally, a total land area of 831 million hectares is salt affected. African countries like Kenya (8.2 Mha), Nigeria (5.6 Mha), Sudan (4.8 Mha), Tunisia (1.8 Mha), Tanzania (1.7 Mha) and Ghana (0.79 Mha) are salt affected to various degrees (FAO, 2000). Salt stress is known to perturb a multitude of physiological processes (Noreen and Ashraf, 2008). It exerts its undesirable effects through osmotic inhibition and ionic toxicity (Munns *et al*., 2006). Increased salinity caused a significant reduction in germination percentage, germination rate, and root and shoots length and fresh root and shoots weights (Jamil *et al*., 2006).

In Ethiopia salt-affected soils are prevalent in the Rift Valley and the lowlands. The Awash Valley in general and the lower plains in particular are dominated by salt-affected soils (Gebreselassie, 1993). A significant abandonment of banana plantation and a dramatic spread to the adjacent cotton plantation of Melka Sadi Farm was reported (Abeaz, 1995). Moreover, of the 4000 ha irrigated land of the above farm 57% has been salt-affected (Taddese and Bekele, 1996). Similarly, the occurrence of salinity problem in Melka Werer Research Farm was reported (Haider *et al*., 1988). Another study also depicted that of the entire Abaya State Farm, 30% has already been salt-affected (Tsige *et al*., 2000).

This problem is expected to be severe in years to come. Because under the prevailing situation of the country; there is a tendency to introduce and implement large-scale irrigation agriculture so as to increase productivity (Mamo *et al*., 1996). In the absence of efficient ways of irrigation water management, salt-build up is an inevitable problem. To alleviate the problem, we need to look for a solution (Gebre and Georgis, 1988). It can be done either using physical or biological practice (Gupta and Minhas, 1993; Marler and Mickelbart, 1993). Since environmental management (physical approach) is not economically feasible (El-Khashab *et al*., 1997) there is a need to concentrate on the biological approach or crop management (Ashraf and McNeilly, 1988; Ashraf *et al*., 2008; Ashraf, 2009). Nevertheless, to proceed with this approach, affirming the presence of genetically based variation for salt-tolerance in a particular crop is a prerequisite (Verma and Yadava, 1986; Marler and Mickelbart, 1993; Mahmood *et al*., 2009).

Therefore, this research will attempt to investigate the salt tolerance of 14 haricot bean (*Phaseolus vulgaris)* varieties for salt tolerance during germination and seedling growth. The reason for selecting haricot bean for this research are being a crop that can be grown in times of intermittent rainfall and also being one of the major export crops of the country (Alemu and Seifu, 2003). Moreover, previous reports on salt tolerance of haricot bean are relatively few.

MATERIALS AND METHODS

This study was conducted in Ethiopia from March 2008 to April 2009 at Mekelle University, in the laboratory of Biology Department. Seeds of fourteen haricot bean (*Phaseolus vulgaris*) varieties were obtained from Melkassa Agricultural Research Center (MARC). The specific haricot bean varieties used in the research were variety Roba-1, Gofta, Chore, Awash-1, Awash Melka, Sinkinesh, Dimtu, Red Kidney (DRK), Tabor, Nassier, Zebra, Cranscope, Mexican 142 and Argene. Moreover, the NaCl concentrations used were 2, 4, 8 and 16 dS/m. These salinity levels were obtained by dissolving 1.12, 2.10, 4.95 and 9.9 g NaCl in one liter of distilled water respectively. Distilled water (0 dS/m) was served as a control.

Germination experiment was conducted in a laboratory, at room temperature using the procedures followed by Verma and Yadava (1986) and Mamo *et al*. (1996). Petri dishes with a diameter of 10 cm that have been lined with whatman No. 3 filter paper were supplied with 10ml of each treatment solution (4 treatment solutions and the control). Following this, eight uniform seeds of each haricot bean varieties were placed on each petri dish and the petri dishes were arranged in a randomized complete block design (RCBD) with four replications. Eventually, the petri dishes were covered with a polyethylene sheet to avoid the loss of moisture through evaporation. Treatment application continued every other day and germination count was started after 48 h of sowing and continued until the 14^{th} day. The seed was considered to have germinated when both the plumule and radicle had emerged >0.5 cm. After the 14 days, overall shoot and the longest root length of six randomly selected seedlings from each replicate were measured using a draftsman ruler. Then their fresh weight was recorded and finally oven dried at 70ºC for 48 h and the seedling dry weight was measured using sensitive balance.

RESULTS

Final Germination Percentage (FGP): The ANOVA for treatments with respect to Final Germination Percentage (FGP) was found to be significant ($p<0.0001$). However, it was insignificant for both varieties and treatment*variety interaction ($p>0.05$). Final germination

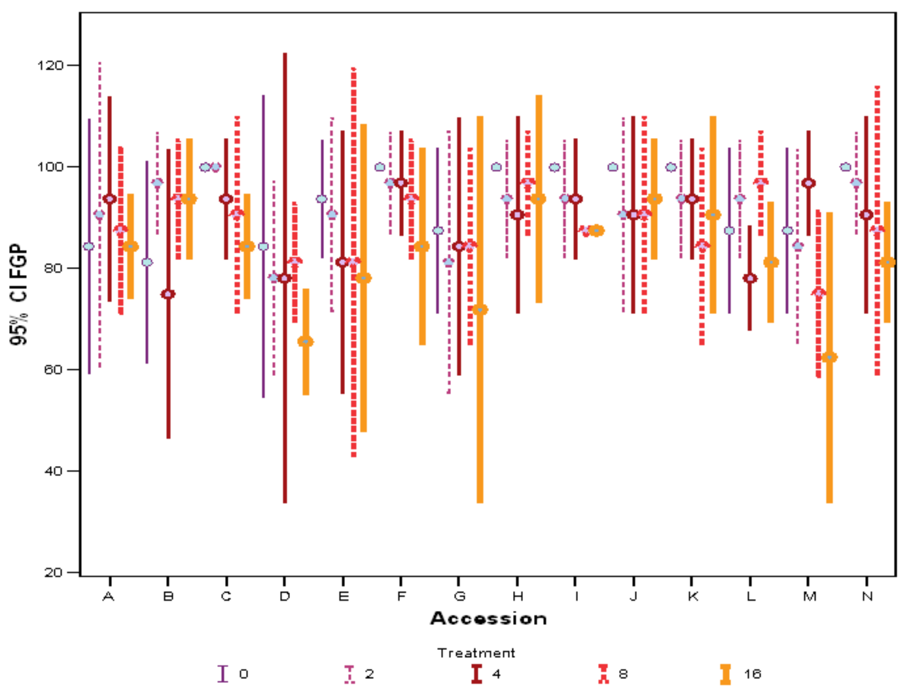

Fig. 1: Effects of different salinity levels (0, 2, 4, 8 and 16 dS/m) on Final Germination Percentage (FGP) of *Phaselolus vulgaris varieties*. (Key to Varieties: R = Roba-1, G = Gofta, C = Chore, A = Awash-1, W = Awash Melka, S = Sinkinesh, D = Dimtu, E = Red Kidney (DRK), T = Tabor, N = Nassier, Z = Zebra, P = Cranscope, M = Mexican 142 and B = Argene)

percentage was facilitated at 2 dS/m in varieties Roba-1, Gofta and Cranscope as compared to the control. At 2, 4 and 8 dS/m salinity levels the effect of salinity was not profound vis-à-vis final germination percentage. However, it was significant at 16 dS/m salinity level (Fig. 1). Varieties Awash 1, Dimtu and Mexican 142 were found to be salt-sensitive but varieties Roba-1, Gofta, Sinkinesh, Red Kideny (DRK) Tabo*r*, Nassier and Zebra were salt-tolerant at 16 dS/m salt concentration. The remaining varieties were found to be intermediate in their response to salt stress with respect to FGP.

Seedling Shoot Length (SSL): The ANOVA for both varieties as well as treatment*variety interaction appeared insignificant ($p>0.05$). Nevertheless, it was significant for treatment ($p<0.0001$). Seedling Shoot Length (SSL) was enhanced at 2dS/m in variety Gofta as compared to the control. Except, this variety, an increment in salt concentration reduced SSL in all varieties. The influence was more pronounced at 16 dS/m salinity level (Fig. 2). Varieties Roba-1, Chore, Awash 1, Red Kidney (DRK), Tabor, Dimtu, Cranscope, Zebra and Argene were found to be salt-sensitive whereas varieties Awash Melka, Mexican 142 and Nassier were found to be salt-tolerant at 16 dS/m salinity level. However, the remaining varieties remained intermediate in their salt tolerance with respect to seedling shoot length.

Seedling Root Length (SRL): The ANOVA for both varieties and variety*treatment interaction was insignificant ($p>0.05$). However, it was significant for treatment ($p<0.0001$). Seedling Root Length (SRL) was facilitated at 2 dS/m in variety Cranscope compared to the control. Generally, 2 and 4 dS/m affected SRL but not significantly from the control. Varieties Roba-1, Red Kidey (DRK), Tabor, Nassier, Dimtu and Zebra were salt-sensitive but varieties Chore, Awash-1, Awash melka, Sinkinesh and Mexican 142 were salt-tolerant at 8 dS/m salinity level (Fig. 3). The remaining varieties were intermediate in their salt- tolerance with respect to Seedling Root Length (SRL).

Shoot-to-Root Ratio (SRR): The ANOVA for treatment was significant ($p<0.0001$). However, it was insignificant for both varieties and variety*treatment interaction ($p>0.05$). In varieties Roba-1, Chore, Awash Melka, Nassier and Argene the value of Shoot-to-Root Ratio (SRR) was high at 16 dS/m as compared to the control. But it was smaller than the control value in varieties Gofta, Awash-1, Red Kidney, Tabor and Cranscope. In the remaining varieties, SRR had almost comparable value at all salinity levels and the control (Fig. 4).

DISCUSSION

Variety* treatment interaction was insignificant for Final Germination Percentage (FGP), Seedling Shoot

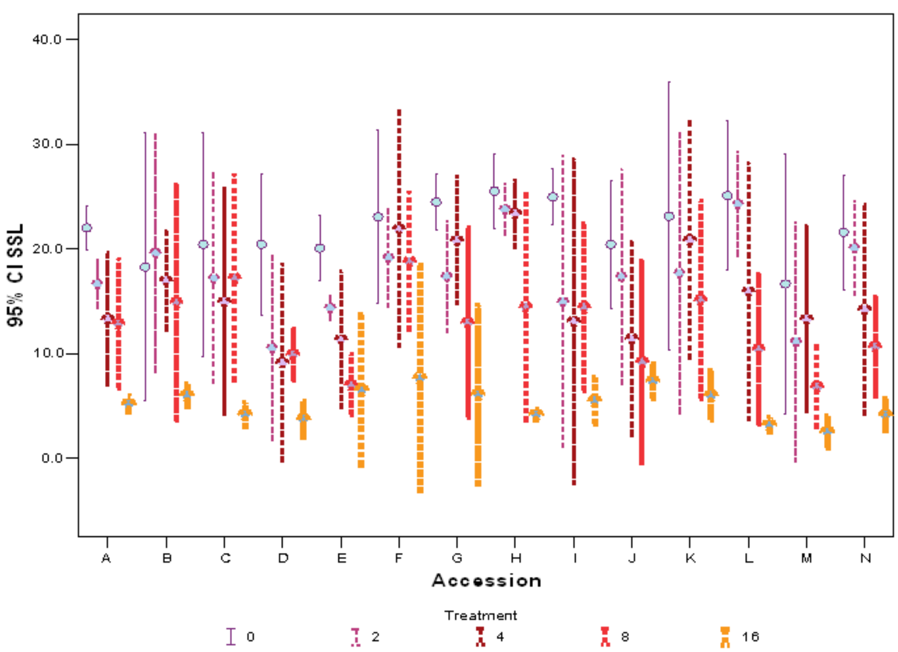

Fig. 2: Effects of different salinity levels (0, 2, 4, 8 and 16 dS/m) on Seedling Shoot Length (SSL) of *Phaselolus vulgaris varieties* (in centimeter). (Key to Varieties: R = Roba-1, G = Gofta, C = Chore, A = Awash-1, W = Awash Melka, S = Sinkinesh, D = Dimtu, E = Red Kidney (DRK), T = Tabor, N = Nassier, Z = Zebra, P = Cranscope, M = Mexican 142 and B = Argene)

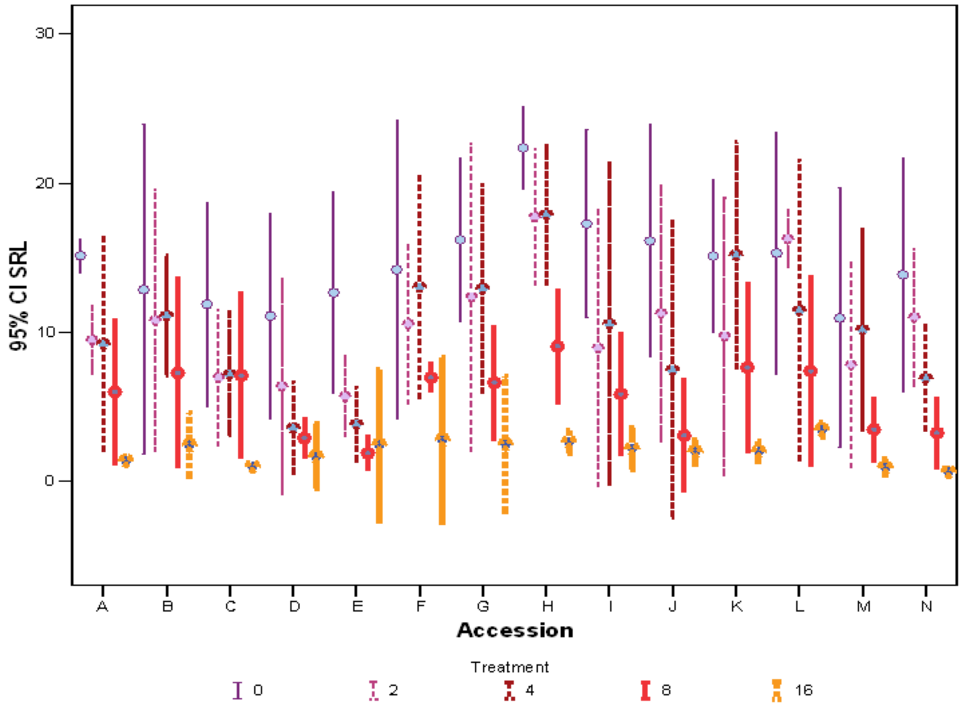

Fig. 3: Effects of different salinity levels (0, 2, 4, 8 and 16 dS/m) on Seedling Root Length (SRL) of *Phaselolus vulgaris varieties* (in centimeter). (Key to Varieties: R = Roba-1, G = Gofta, C = Chore, A = Awash-1, W = Awash Melka, S = Sinkinesh, D = Dimtu, E = Red Kidney (DRK), T = Tabor, N = Nassier, Z = Zebra, P = Cranscope, M = Mexican 142 and B = Argene)

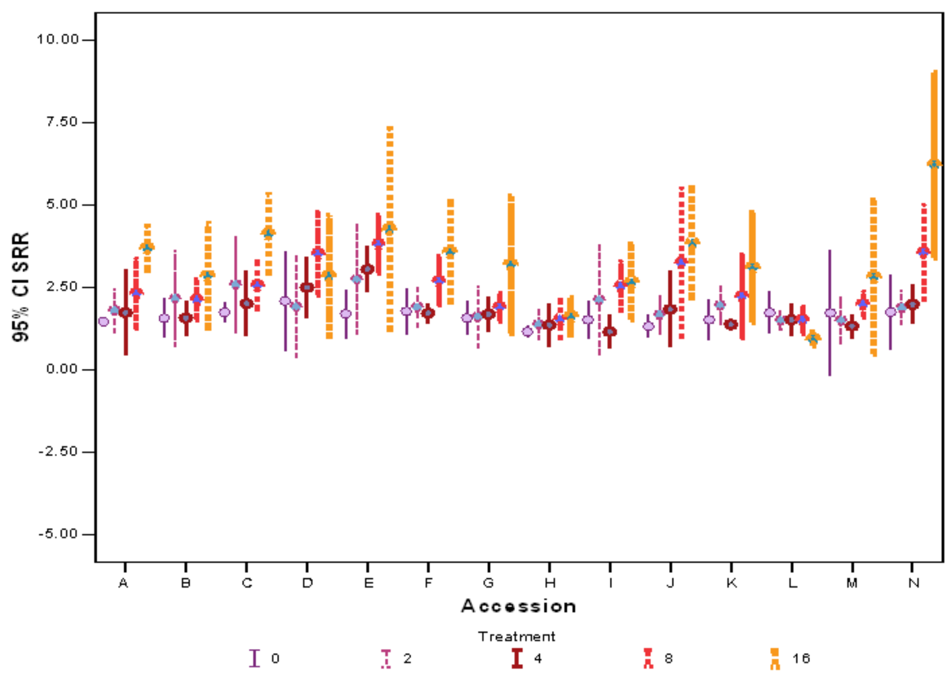

Fig. 4: Effects of different salinity levels (0, 2, 4, 8 and 16 dS/m) on Shoot-to-Root Ratio (SRR) of *Phaselolus vulgaris varieties*. (Key to Varieties: R = Roba-1, G = Gofta, C = Chore, A = Awash-1, W = Awash Melka, S = Sinkinesh, D = Dimtu, E = Red Kidney (DRK), T = Tabor, N = Nassier, Z = Zebra, P = Cranscope, M = Mexican 142 and B = Argene)

Length (SSL), Seedling Root Length (SRL) and seedling Shoot-to-Root-Ratio (SRR) during germination and seedling growth reflecting that all varieties responded similarly to salt stress with respect to the above parameters. In general, salt stress at 2 dS/m has enhanced growth with respect to FGP, SSL, SRL and SRR in some varieties. The impact of 2 and 4 dS/m salinity levels was not profound with respect to all parameters considered. Nevertheless, most varieties were quite salt-sensitive at 16 dS/m salinity level.

The ANOVA for treatments was significant with regard to all parameters considered. On the one hand, the ANOVA for varieties was insignificant with respect to FGP, SSL, SRL and SRR. This implies that there was no significant varietal difference among haricot bean varieties in relation to the parameters considered. Increment in salinity level caused reduction in Final Germination Percentage (FGP) in most varities but the drop was quite sharp and rapid in varieties Awash 1, Dimtu and Mexican 142 at 16 dS/m salinity level. Similar results were reported in California mariout barley, 'salina' strawberry, clover and Landino clover (George and Williams, 1964), triticales (Norlyn and Epstein, 1984), wheat and durum wheat (Francois *et al*., 1986), oats (Verma and Yadava, 1986), rice (Heenan *et al*., 1988; Lee *et al*., 1998), alfalfa (Al-Neimi *et al*., 1992), chickpea and lentil, durum wheat and tef (Mamo *et al*., 1996), cowpea (Murillo-Amador and Troyo- Die'Guez, 2000), sorghum (Geressu and Gezahagne, 2008).

In all varieties studied, seedling mortality aggravated as the salinity level gets maximized. Similar results were reported in rice (Lee and Senadhira, 1998), pigeonpea (Subbarao *et al*., 1990) and cotton seedlings (Lin *et al*., 1997). Crop cultivar may germinate effectively under salt stress; nevertheless, its seedling growth may be affected (Azhar and McNeilly, 1987; Francois *et al*., 1989; Miyamoto, 1989). In line with this, varieties Roba-1, Red kidney (DRK), Tabor and Zebra were less salt-affected during germination than later growth (had inadequate seedling growth). This implies that these varieties are salt-tolerant during germination than subsequent growths like seedling growth. On the other hand, crop genotype may be salt-sensitive during germination and seedling growth. It has already been reported in Balansa clover and subterranean clover (Rogers and Noble, 1991), cowpea (Murillo-Amador and Troyo-Die'guez, 2000), rice (Shannon *et al*., 1998), oats (Verma and Yadava, 1986; Ashraf and Waheed, 1992). Similarly, in this research, variety Dimtu was found salt-sensitive at higher salinity levels during germination and seedling growth. Thus this haricot bean variety can not be cultivated even on slightly saline soils.

Contrary to this, Awash Melka was salt-tolerant during germination and seedling growth. Its extraordinary salt-tolerance ability might be achieved through reduced respiration (George and Williams, 1964), dilution of toxic effects of salts (Lee and Senadhira, 1998), and accumulation of compatible solutes (Kayani *et al*., 1990). It has already been reported that plant growth and development is dependent on crop stand establishment (Verma and Yadava, 1986), in turn, the latter is a function of effective germination (Horst and Taylor, 1983) and seedling growth (Ashraf and Waheed, 1992). Crops with higher germination percentage can establish themselves effectively on moderately saline soils (Lee *et al*., 1998).

Thus even if, it is difficult to extrapolate the research result obtained from controlled experiment directly to the field, relatively the above haricot bean variety which had the highest Final Germination Percentage (FGP) could germinate and establish itself effectively on moderately saline soils. Thus as Ashraf and McNeilly (1988) pinpointed such salt-tolerant variety could be used in three ways. Namely, for direct cultivation on moderately saline areas (provided that it is adapted to various environmental situations), to produce more salt tolerant lines through breeding and finally to uncover important genes with enhanced salt tolerance. Consequently, this would help to minimize grain yield loss that could emanate from inadequate crop stand establishment as a result of poor germination and seedling growth.

CONCLUSION

Seedling Root Length (SRL) was more salt affected than Seedling Shoot Length (SSL) in the fourteen haricot bean varieties studied. Lower salinity levels such as 2 and 4 dS/m have facilitated germination and seedling growth in some haricot bean varieties. The rate of seedling mortality showed increment as the salinity levels become higher and higher. The impact of salinity on haricot bean varieties became intense upon salinity level increment and growth stage advancement. Varieties Roba-1, Red kidney (DRK), Tabor and Zebra were salt sensitive during seedling growth. Moreover, variety Dimtu was salt-sensitive during both at germination and seedling growth. Thus these varieties can not be cultivated even on slightly saline soils. However, variety Awash Melka was salt-tolerant during germination and seedling growth. So it can be cultivated effectively on moderately saline soils.

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