Effect of NaCl on the productivity of four selected common bean cultivars (Phaseolus vulgaris L.)

Patrick A. Ndakidemi* and Joachim H. J. R. Makoi

Faculty of Applied Science, Cape Peninsula University of Technology, Cape Town Campus, Keizersgracht, P. O. Box 652, Cape Town 8000, South Africa.

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Four cultivars of bean (Lyamungo 90, Jesca, Flora de Mayo and CAB 19) were tested under differing NaCl concentration to assess their performance in a salt rich medium. The aim was to select salt tolerant bean cultivars. The seeds of these cultivars were inoculated with rhizobium strain (Rhizobium leguminosarum biovar phaseoli) and grown in the greenhouse. After the establishment of the seedlings, plants were administered with doses of saline solutions at varying concentrations of NaCl (0, 2.5, 5.0, 10.0 mM) and collected 10 and 20 days after NaCl treatment. Results showed that higher NaCl concentrations reduced plant height, dry matter yield and also altered the leaf colour and promoted their leaf injury. The more severely affected bean cultivars were Lyamungo 90 and CAB 19 at > 5.0 mM NaCl compared to the control. Cultivars Jesca and Flora de Mayo showed better results in these parameters than cultivars Lyamungo 90 and CAB 19. The results suggest that some cultivars are more tolerant to high salinity than others; those tolerant cultivars can sustain plant growth and exhibit less signs of leaf injury and colour loss when compared to more susceptible cultivars in high salinity conditions.

Key words: Leaf color damage, leaf injury, Phaseolus vulgaris, plant cultivars, salt stress.

INTRODUCTION

Soil salinity is one of the major limiting factors to crop productivity in many arid and semi-arid areas in the world (Fischer and Turner, 1978). Salinity is common in arid and semi-arid areas where potential evapotranspiration exceeds annual precipitation. Under such conditions, major cations (Na\(^+\), Ca\(^{2+}\), Mg\(^{2+}\), K\(^+\)) and anions (Cl\(^-\), SO\(_4\)^\(^{2-}\), HCO\(_3\)^\(^-\) and NO\(_3\)^\(^-\)) accumulate in the soil to high levels which can lower crop productivity (Spark 1995). It is from this background that one can declare soil salinity as the largest constraint to plant productivity and specifically to global food production (Pitman and Läuchli, 2002).

Under normal circumstances, farmers would wish to produce their crops in good soils that are rich in mineral nutrients. Recent research has shown that the human population in most areas with suitable soils, such as those found in Africa, has increased at an alarming rate (Smaling et al., 1997; Pimentel et al., 1999). Farmers have now extended their farming activities to the lowlands where low or unreliable and erratic rainfall coupled with high temperature and evaporation favours the accumulation of salts in varying concentrations (Ebert et al., 2002). In order to economically exploit these areas, it is important to search for crop plants that will thrive in such soils and produce reasonable yields.

The common bean (Phaseolus vulgaris L.) is amongst the most important and valuable leguminous crops in the semi-arid parts of Africa. Besides providing high quality protein for human consumption, P. vulgaris also contributes to the sustainability of traditional cropping systems in which small scale farmers predominate (Long, 1984; Herridge et al., 1995). In most African countries, P. vulgaris L. is cultivated in soils with no or low saline content. With the current trend in population increase, bean growers are expanding productivity to lowland areas where in some cases the salinity is one of the abiotic constraints. From this background, it is important to search for bean cultivar(s) in the African continent and elsewhere that will survive and produce reasonable yields at different levels of salt concentrations, such as those found in natural environments.
It has been shown that salt stress may lead to decreased dry matter yield (Hernandez et al., 1995; Cherian et al., 1999; Takemura et al., 2000), water and osmotic stress, decreased chlorophyll content and gas exchange variables (Agastian et al., 2000; Kyoro, 2006), ions and mineral elements imbalance (Khan, 2001) as well as several other negative impacts on physiological processes in the plant. However, legume cultivars behave differently under saline conditions (Zahran, 1991). Their response to stress is dependent on a number of factors including the concentration of salt in soil solution (Cordovilla et al., 1995) and the genetic make up of the plant (Singh et al., 2008). Although the effect of salinity on the growth of some crop plants has been studied extensively (Lauter and Munns, 1986; Elsheikh and Wood, 1990; George et al., 1997; Heuperman, 1999), very little is known of the effect of different salinity concentrations on growth, leaf colour changes and foliar injury in different bean cultivars developed by various national bean programs in Africa. Testing the selected higher yielding bean cultivars for their tolerance to a salt stressed environment would be one way to exploit their growth potential and to economically utilise the salt affected areas in Africa. This study seeks to evaluate the effects of different NaCl concentrations on growth, dry matter yield, leaf injury and colour changes in the selected four cultivars of bean (Lyamungu 90, Jesca, Flora de Mayo and CAB 19) from the national bean program in Tanzania for use in salt affected areas. Any positive results will be a step towards identifying genotype(s) with a salt tolerance mechanism.

MATERIALS AND METHODS

Site description

The experiment was conducted during the summer of 2007 in the research greenhouse at the horticultural unit of the Cape Peninsula University of Technology (CPUT), Cape Town, South Africa. Soil material was collected from the Nietvoorbij Agricultural Research Council (ARC) station, Stellenbosch, South Africa. The soil used for this study is a sandy loam classified as skeletal leptosol in the Food and Agriculture Organization (FAO) soil classification system (SCWG, 1991). The soil was sampled randomly (0 – 15 cm) from an area of 1 ha. After transferring the soil to the greenhouse, 2.0 kg soil was carefully packed into 2 kg pots. Prior to the onset of the experiment, sub-samples were analysed for chemical properties (Table 1).

Experimental layout and salt treatment

Bean cultivars used in the experiment were obtained from the breeder at Selian Research Agricultural Institute (SARI), Arusha, Tanzania. They included: Lyamungu 90, Jesca, Flora de Mayo and CAB 19. Their selection was based on their excellent growth potential of NaCl. The pots were arranged in a randomized complete block design in the greenhouse and replicated 4 times. Prior to planting, all seeds were inoculated with rhizobium strain (Rhizobium leguminosarum biovar phaseoli). NaCl treatment was initiated 10 days after sowing. The average temperature in the greenhouse was 20°C/15°C day/night.

Seed germination and growth

3 seeds of the selected cultivars were sown in each pot and these were thinned to 2 plants 10 days after sowing. After germination and during the growing period, the seedlings were frequently irrigated with water previously prepared with different molar concentrations of NaCl salt. Moisture content was monitored regularly by supplying the plants with equal amounts of water to ensure that the pots were not moisture deficient.

Growth measurements and sampling

Measurements across all cultivars commenced 20 days after planting, that is, 10 days after salt treatment and every 10 days of NaCl treatment thereafter. At each measuring time, plants were up-rooted and separated into roots and shoots. Roots were washed in tap water and rinsed in de-ionised water. Roots and shoots were oven dried at 60°C for 48 h. Dry root and shoot biomass were recorded. The leaf colour changes and leaf injury from salt damage were assessed on a scale of 1 - 5. These were based on other related assessments by Fleming and Palmer (1975), Rodriguez de Cianzio et al. (1979), Xu et al. (2000) and Maher et al. (2003). The scale of 5 indicated healthy leaves with green colour and the scale of 1 represented severely chlorotic and injured leaves. The proposed scale enabled us to estimate the extent of colour changes and tissue damage as expressed by different bean cultivars at varying NaCl concentrations.

Statistical analysis

Statistical analysis of data collected was performed by One Way ANOVA using STATISTICA program 2008 (StatSoft Inc., Tulsa, OK, USA). Where F-value was found to be significant, Fisher’s least significant difference (LSD) was used to compare the means at P ≤ 0.05 (Steel and Torrie, 1980).

RESULTS

Effects of different NaCl concentrations on plant height in different bean cultivars

Plant height measured 10 days after NaCl treatment was significantly (p ≤ 0.001) influenced by the treatments only in the Lyamungu 90 bean cultivar (Table 2). As compared with the control and the 2.5 mM NaCl, significantly poor growth was noted in treatments supplied with 5.0 and 10.0 mM NaCl (Table 2). In contrast, plant height in other bean cultivars tested (Jesca, Flora de Mayo and CAB 19) was not significantly affected by the NaCl treatment after the 10 days. Repeated measurements taken 20 days after the NaCl treatment revealed that plant height was...
Table 1. Chemical properties of soil used in pots at planting.

<table>
<thead>
<tr>
<th>pH (CaCl₂)</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>S</th>
<th>Na</th>
<th>Fe</th>
<th>Cu</th>
<th>Zn</th>
<th>Mn</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg.kg⁻¹</td>
<td>µg.g⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.15</td>
<td>18.56</td>
<td>136.57</td>
<td>68.70</td>
<td>15.50</td>
<td>3.97</td>
<td>90.44</td>
<td>255.90</td>
<td>3.59</td>
<td>3.19</td>
<td>8.62</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Table 2. Effect of increasing NaCl concentrations on plant height (cm) in different bean cultivars.

<table>
<thead>
<tr>
<th>Treatment NaCl (mM)</th>
<th>Measurement taken 10 days after salt treatment and 20 days after planting</th>
<th>Measurement taken 20 days after salt treatment and 30 days after planting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LYAMUNGO 90</td>
<td>JESCA</td>
</tr>
<tr>
<td>0</td>
<td>29.9 ± 0.9a</td>
<td>24.1 ± 2.0a</td>
</tr>
<tr>
<td>25</td>
<td>27.8 ± 1.7a</td>
<td>24.0 ± 1.1a</td>
</tr>
<tr>
<td>50</td>
<td>23.5 ± 1.0b</td>
<td>23.8 ± 1.0a</td>
</tr>
<tr>
<td>100</td>
<td>19.0 ± 0.6c</td>
<td>21.0 ± 2.3a</td>
</tr>
</tbody>
</table>

F-Statistic 18.8*** 0.743 ns 2.5 ns 9.9** 7.1** 13.9*** 7.6**

** = p ≤ 0.01; *** = P ≤ 0.001; ns = Not significant.

Values (Mean ± SE) followed by dissimilar letters in the same column are significant at P ≤ 0.05 according to Fischer LSD.

significantly changed with different NaCl treatments in all bean varieties. For instance, increasing NaCl concentration to 2.5 mM NaCl did not significantly change the plant height in Lyamungu 90. In the other 3 cultivars plant height was significantly reduced at NaCl concentration of 10.0 mM.

**Effect of different NaCl concentrations on root and shoot biomass of different bean cultivars**

Increasing NaCl concentrations significantly (p ≤ 0.05) decreased root biomass in cultivars Lyamungo 90 and Flora de Mayo for samples collected 10 days after NaCl treatment (Table 3) and in cultivars Jesca and CAB 19 for data collected 20 days after NaCl treatment (Table 3). Generally, a significant weight reduction in root biomass was observed at the highest NaCl concentration compared with the control treatment (Table 3).

Data on shoot biomass collected at 10 days after NaCl treatments showed no significant differences (Table 4). However, at 20 days after NaCl treatment, shoot biomass was significantly reduced by higher NaCl concentrations compared to the control treatment (Table 4). Across all cultivars, lowest values of shoot biomass were recorded in the treatment involving 10.0 mM NaCl (Table 4).

**Effects of different NaCl concentrations on leaf colour damage in different bean cultivars**

The influence of different concentrations of NaCl on leaf colour for the different cultivars are presented in Table 5. Measurements collected 10 days after the NaCl treatments indicated that not all cultivars exposed to different NaCl concentrations were significantly affected. Colour changes were not significantly influenced by NaCl treatments in Lyamungo 90 and Jesca. However, leaf colour measured 10 days after NaCl treatment in Flora de Mayo and CAB 19 was significantly changed by NaCl treatments. In Flora de Mayo, the concentration of NaCl up to 5.0 mM did not significantly cause colour damage as compared to supplying this variety with 10.0 mM. In bean cultivar CAB 19, leaf colour damage was observed to increase progressively at each level of NaCl supply, reaching maximum damage at the highest NaCl concentration of 10.0 mM.

For colour measurements taken at 20 days after salt treatment, significant adverse effects of salinity were recorded in all bean cultivars tested. As compared with the control, supplying NaCl at 2.5 mM induced significant small colour changes in all bean cultivars. Moreover, cultivars Jesca and Flora de Mayo were slightly tolerant of NaCl damage up the concentration of 5.0 mM as compared to other bean cultivars when judged by leaf colour changes. In this study, colour damage in all cultivars tested was more pronounced in the treatments involving 10.0 mM NaCl.

**Effects of different NaCl concentrations on leaf injury in different bean cultivars**

Results on leaf injury are shown in Table 6. Foliar injury was not observed in the control treatments (0 mM NaCl). In contrast, most treatments supplied with NaCl showed visible foliar injuries which were very predominant on leaf margins and leaf tips. The reaction of bean cultivars was quite different. For instance, data collected from cultivars...
Table 3. Effect of different NaCl concentrations on root dry weight (g) in different bean cultivars.

<table>
<thead>
<tr>
<th>Treatment NaCl (mM)</th>
<th>Measurement taken 10 days after salt treatment and 20 days after planting</th>
<th>Measurement taken 20 days after salt treatment and 30 days after planting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LYAMUNGO 90</td>
<td>JESCA</td>
</tr>
<tr>
<td>0</td>
<td>0.12 ± 0.02a</td>
<td>0.08 ± 0.01a</td>
</tr>
<tr>
<td>25</td>
<td>0.09 ± 0.01ab</td>
<td>0.07 ± 0.01a</td>
</tr>
<tr>
<td>50</td>
<td>0.06 ± 0.01b</td>
<td>0.06 ± 0.01a</td>
</tr>
<tr>
<td>100</td>
<td>0.06 ± 0.01b</td>
<td>0.04 ± 0.01a</td>
</tr>
</tbody>
</table>

F-Statistic | 4.874* | 2.882 ns | 3.834* | 0.296 ns | 1.550 ns | 3.410* | 2.354 ns | 12.960*** |

* = p < 0.05; *** = p < 0.001; ns = Not significant
Values (Mean ± SE) followed by dissimilar letters in the same column are significant at P ≤ 0.05 according to Fischer LSD.

Table 4. Effect of different NaCl concentrations on shoot dry weight (g) in different bean cultivars.

<table>
<thead>
<tr>
<th>Treatment NaCl (mM)</th>
<th>Measurement taken 10 days after salt treatment and 20 days after planting</th>
<th>Measurement taken 20 days after salt treatment and 30 days after planting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LYAMUNGO 90</td>
<td>JESCA</td>
</tr>
<tr>
<td>0</td>
<td>0.42 ± 0.01a</td>
<td>0.39 ± 0.03a</td>
</tr>
<tr>
<td>25</td>
<td>0.36 ± 0.03a</td>
<td>0.37 ± 0.02a</td>
</tr>
<tr>
<td>50</td>
<td>0.35 ± 0.02a</td>
<td>0.35 ± 0.04a</td>
</tr>
<tr>
<td>100</td>
<td>0.29 ± 0.06a</td>
<td>0.32 ± 0.03a</td>
</tr>
</tbody>
</table>

F-Statistic | 2.286 ns | 1.108 ns | 0.855 ns | 0.467 ns | 14.473*** | 8.766** | 19.440*** | 8.176** |

** = P ≤ 0.01; *** = P ≤ 0.001; ns = Not significant
Values (Mean ± SE) followed by dissimilar letters in the same column are significant at P ≤ 0.05 according to Fischer LSD.

Jesca and Flora de Mayo 10 days after NaCl treatments showed no significant visible injury symptoms on their leaves. In contrast, Lyamungu 90 and CAB 19 exposed to 10.0 mM NaCl were significantly injured by NaCl treatments.

NaCl treatments in the study significantly increased leaf injury in all bean cultivars tested 20 days after salt treatment. The complete injury in Lyamungu 90 and Jesca was recorded in treatments supplied with 10.0 mM NaCl. Contrarily, in Flora de Mayo and CAB 19, complete injury was recorded at the concentration of 5.0 and 10.0 mM NaCl. Generally, severely injured leaves showed necrotic lesions on the leaf blade and later the leaf lamina rolled towards the midrib, which resulted in the death of the plant.

**DISCUSSION**

In this study, the effect of varying NaCl concentration on growth (plant height and dry matter yield), leaf colour changes and foliar injury in four common bean (P. vulgaris) cultivars (Lyamungo 90, Jesca, Flora de Mayo and CAB 19) was assessed. Results have shown that exposure of different bean cultivars to increasing NaCl concentrations initially decreased (p ≤ 0.05) plant height in cultivar Lyamungo 90 but not cultivars Jesca, Flora de Mayo and CAB 19. However, plant height was decreased with all cultivars as the salt stress was increased in measurements taken at 10 and 20 days after NaCl treatments (Table 2). The decreased plant height under high salt stress (10.0 mM NaCl) as observed in this study suggests a toxic effect from high ion concentration in different plant tissues. Consistent with these results, previous studies have shown that plant height and dry matter yield in legumes such as P. vulgaris were reduced under salt stress (Delgado et al., 1994; Demir; Kocacaliskan, 2002). Also, different legumes showed differences in sensitivity and tolerance to various salt concentrations in salt affected mediums (Lauchli, 1984; Elsheikh and Wood, 1990; Rumbaugh et al., 1993) as shown in this study. Collected data showed that cultivar Lyamungo 90 was more sensitive to increased salt stress compared to the other cultivars, suggesting that Jesca, Flora de Mayo and CAB 19 were initially able to adjust to salt stress, probably by lowering ion toxicities in their tissues (Table 2; Munns, 2002; Parida and Das, 2005) which would result in the observed improved plant growth as reported in our study.

As shown in Tables 3 and 4, higher NaCl concentrations decreased root and shoot biomass in some of the tested cultivars. For example, sampling done 10 days after salt...
Table 5. Effect of different NaCl concentrations on leaf colour of different bean cultivars.

<table>
<thead>
<tr>
<th>Treatment NaCl (mM)</th>
<th>Measurement taken 10 days after salt treatment and 20 days after planting</th>
<th>Measurement taken 20 days after salt treatment and 30 days after planting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LYAMUNGO 90</td>
<td>JESCA</td>
</tr>
<tr>
<td>0</td>
<td>5.0 ± 0.00a</td>
<td>5.0 ± 0.00a</td>
</tr>
<tr>
<td>25</td>
<td>4.0 ± 0.00a</td>
<td>4.0 ± 0.00a</td>
</tr>
<tr>
<td>50</td>
<td>3.0 ± 0.00a</td>
<td>4.0 ± 0.00a</td>
</tr>
<tr>
<td>100</td>
<td>3.0 ± 0.00a</td>
<td>3.0 ± 0.00a</td>
</tr>
<tr>
<td>F-Statistic</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

** = p ≤ 0.01; *** = p ≤ 0.001; ns = Not significant

Values (Mean ± SE) followed by dissimilar letters in the same column are significant at P ≤ 0.05 according to Fischer LSD.

Treatment showed that increasing NaCl concentrations did not reduce the root biomass of Jesca and CAB 19 cultivars, whereas those of Lyamungo 90 and Flora de Mayo were significantly affected (Table 3). However, opposite results were recorded in sampling done 20 days after NaCl treatment. Only the root biomass of cultivars. Jesca and CAB 19 were significantly changed with NaCl treatments (Table 3). With regard to shoot biomass, NaCl had no significant influence across all bean cultivars for the sampling done 10 days after NaCl treatment (Table 4), but further exposure of these cultivars to NaCl treatment for a period of 20 days significantly reduced shoot biomass at higher NaCl concentrations (Table 4). The decrease in root and shoot biomass at varying periods and stress sensitivity levels between the bean cultivars suggests that Na⁺ and/or Cl⁻ toxicities in the tissues could have inhibited root elongation, food synthesis and its translocation to the growing shoots. This might have been the cause of low root and shoot biomass in plants exposed to higher salt concentrations (Tables 3 and 4, Cordovilla et al., 1995; Mohamed et al., 1998; Meloni et al., 2001). Parallel to these results, reduced root and shoot biomass as a result of higher NaCl concentration have also been reported in other studies involving chickpeas (Cicer arietinum L.), field peas (Pisum sativum L.), faba bean (Vicia faba L.) and common bean (Phaseolus vulgaris L.) (Maas and Grieve, 1987; Elsheikh and Wood, 1990; Brugnoli and Lauteri, 1991; Delgado et al., 1994; Soussi et al., 1998; Munns, 2002).

Leaf colour was significantly affected in all the bean cultivars as a result of increased NaCl concentration (Table 5), the effects being most pronounced in cultivars Lyamungo 90 and CAB 19 at higher NaCl concentration. Increased stress due to higher NaCl concentrations led to the significant damage to the leaf colour pigments (Table 5). Similar results have been reported in other legumes (Soussi et al., 1998; Al-Khanjari et al., 2002). The leaf colour damage observed in those plants supplied with higher levels of NaCl concentrations could be due to suppression of specific enzymes responsible for the synthesis of green pigments (Strogonove et al., 1970; Shi and Zhao, 1997; Sudhakar et al., 1997), an effect that depends on the biological processes and developmental stages of the plant as well as the type and concentration of the NaCl. The lower reduction in leaf colour and less leaf damage in the tolerant cultivars. Jesca and Flora de Mayo might have been responsible for their higher dry matter yield. However, more work is required to establish mechanism(s) involved in the survival of these cultivars in saline environments.

Increasing NaCl concentration led to leaf injury in the cultivars tested (Table 6). However, there were differences in the extent of leaf injury between the cultivars. Generally, leaves of cultivar Jesca were more tolerant of higher salt concentration compared to the other varieties tested. The observed differences in leaf injury between bean cultivars under salt stress conditions clearly indicates potential for genotypic differences between bean germplasm. Generally, leaf injury increased considerably at the highest NaCl treatments. Comparing our results to the control treatment, it is reasonable to speculate that the greater damage observed in higher NaCl treatments was caused by toxic effects of either Na⁺ or Cl⁻ from the salt that was supplied. These results are similar with the previous studies in which higher concentrations of NaCl in the growth media resulted in leaf injury in leguminous crops (Delgado et al., 1994; Tejera et al., 2005, 2006).

Conclusion

In conclusion, the present investigation shows that the induced decrease in plant height, dry matter yield, leaf color and injury was mainly due to an increased salt stress which could have caused the damage of the leaf colour pigments, leading to poor growth. Similarly, plants exposed to high salt stress showed more severe symptoms of leaf injury than those exposed to low NaCl, suggesting that the effects may be due, at least in part, to a direct effect of Na⁺ and/or Cl⁻ toxicities supplied as treatments in this study. The observed differences in leaf injury along with other growth variables (leaf colour, plant
Table 6. Effect of different NaCl concentrations on leaf injury of different bean cultivars.

<table>
<thead>
<tr>
<th>NaCl (mM)</th>
<th>Measurement taken 10 days after salt treatment and 30 days after planting</th>
<th>Measurement taken 20 days after salt treatment and 30 days after planting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LYAMUNGO 90</td>
<td>JESCA</td>
</tr>
<tr>
<td>0</td>
<td>5.0 ± 0.00a</td>
<td>5.0 ± 0.00a</td>
</tr>
<tr>
<td>25</td>
<td>5.0 ± 0.00a</td>
<td>5.0 ± 0.00a</td>
</tr>
<tr>
<td>50</td>
<td>5.0 ± 0.00a</td>
<td>5.0 ± 0.00a</td>
</tr>
<tr>
<td>100</td>
<td>3.3 ± 0.25b</td>
<td>5.0 ± 0.00a</td>
</tr>
<tr>
<td>F-Statistic</td>
<td>49.0***</td>
<td>ns</td>
</tr>
</tbody>
</table>

** = P ≤ 0.01; *** = P ≤ 0.001; ns = Not significant

Values (Mean ± SE) followed by dissimilar letters in the same column are significant at P ≤ 0.05 according to Fischer LSD.

height and dry matter yield) between the bean cultivars tested under salt stress clearly indicates potential genotypic differences between the bean cultivars. In this study, cultivars Jesca and Flora de Mayo showed promising salt stress tolerance in salt affected environments. Detailed studies to establish mechanisms of tolerance in these cultivars are recommended.

ACKNOWLEDGEMENTS

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REFERENCES


