The Effect of Salinity (NaCl) on Germination of Selected Grass pea
(Lathyrus sativus L.) Landraces of Tigray

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Abstract: Worldwide, salinity affects 100 million ha of arable lands and this area is expanding. In this study, a pot experiment was conducted to assess the effect of different salinity levels on germination and seedling growth of grass pea (Lathyrus sativus) landraces collected from Degua, Tembien district. During the experiment, a salt of sodium chloride was used as sources of treatment at different rate of concentrations and seeds were allowed to germinate in different concentration of NaCl. Five levels of NaCl salinity levels: 0 (control), 4, 6, 8 and 10 dS/m, respectively were prepared to simulate different salinity level. And germination percentage, germination index, relative germination rate, relative sodium chloride injury rate were determined for each landraces. All growth attributes such as fresh and dry shoot weights, shoot length and root length decreased with an increase in salinity levels. Furthermore, the landraces from Melfa were found to be highly tolerant to high salinity level. Finally, salinity has a strong effect on germination of Lathyrus sativus with the different selected land races of Tigray and it was concluded that with increase in salinity levels there was a significant reduction in biomass production in grass pea.

Key words: Germination and shoot length, grass pea tigray, salinity

INTRODUCTION

Background of the study: Growth of the human population by 50%, from 6.1 billion in mid 2001 to 9.3 billion by 2050 (FAO, 2004; Nigusse, 2007), means that crop production, must increase if food security is to be ensured, especially for poor people, through different mechanisms. Furthermore, the population of some developing countries including Ethiopia is expected to double in the coming 20 years which creates main challenge of how to double the food production in the next 25 years to feed the rapidly growing population and thereby reduce food scarcity (FAO, 2004). Many least developed countries, including Ethiopia, apply irrigation as an important means of achieving food self-sufficiency (Nigusse, 2007). Increasing the availability of irrigation and less dependency on rain-fed agriculture is taken as a means to increase food production and self-sufficiency of the rapidly increasing population of Ethiopia. As a result irrigated agriculture is at the heart of the agricultural development-led industrialization and food security strategy of the Ethiopian Government (Eyasu, 2005). In line with the development policy of Ethiopian government, the regional government of Tigray has been engaged in earthen dam irrigation development activities for the past decade with a principal objective to change the agrarian system to widespread irrigated agriculture and to gradually attain self-sufficiency in food production. Unfortunately, a strong link with salinization throws an immediate question over the sustainability of using irrigation to increase food production. And it has been argued elsewhere (Flower and Yeo, 2004)that increasing the salt tolerance of crops will be of primary value to the sustainability of irrigation.

Given the amount by which food production will have to be increased in the coming 5 decades, it seems reasonable to predict that changing the salt tolerance of crops will be an important aspect of plant breeding in the future, if global food production is to be maintained (O’ Leary, 1995).

Historically, Lathyrus sativus is one of the ancient crops in Ethiopia. Archaeological evidence from lalibela caves indicated Lathyrus sativus seeds were used for a long period of time for its edible seeds and folder at medium altitudes up to 2200 m mainly in the high lands of north and east of the rift valley. Besides, it is found in Eurasia, North America, temperate South America and rift valley areas of East Africa (Smart, 1990). Lathyrus sativus is particularly well suited to grow on residual moisture in heavy black clay soils and often planted after crops are harvested. In times of rainfall shortage this can be the main crop harvested Tigray is one of the grass pea grower region in Ethiopia next to Adet (Amhara-region) in times of early summer to increase land fertility and for the sufficient food production purpose. Although grass pea like other leguminous crops are widely cultivated and

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consumed by local farmers in Tigray, information on the characteristics of salinity tolerance of the crop is very scarce. This shows the need to study characteristics of indigenous genotypes that can guarantee the good yield of the crop under the saltier arable irrigated lands of Tigray. 

Statement of the problem: Worldwide, salinity affects 100 million ha of arable lands and this area is expanding (Ghassemi et al., 1995). The situation in Tigray is not different from the global trend. Recent studies in the irrigated fields of Mekelle plateau (Enderta Wereda and Hintalo-Wajarat woreda), in Tigray region by Fassil (2008) indicated that irrigated land from 9 dams evaluated in the study are experiencing moderate salinity hazard: May Gassa (EC = 2.56), Adikenafiz (EC = 2.04), Grashito (EC = 3.54) Durambessa (EC = 2.51), Ghereb segen (EC = 3.11) and Gumselasa (EC = 2.35). This indicates irrigated lands in the semi arid parts of the region are increasingly becoming saltier and turning to a new scenario of hampering food production for the fast growing population in the region. And with most arable lands of the region becoming saltier more work on the screening salt tolerance land races should be done since further screening for salinity from their well developed environment could be a guarantee to solve the foreseeable future problem of salinity due to irrigation practice.

Thus, the aim of this study was to investigate the effect of salinity on germination and growth of grass pea landraces and explore the potential salinity tolerance of Tigray grasspea landraces collected from different major agro ecologies of the region.

Objective of the study:

General objective:

To assess the effect of salinity on germination of *Lathyrus sativus* landraces of Tigray collected from different *Lathyrus sativus* growing areas.

Specific objectives:

- To investigate the morphological and physiological traits of *Lathyrus sativus* land races that are potentially related to salinity tolerance.
- To screen salt tolerant *Lathyrus sativus* land races cultivated at different agro-ecological areas of Tigray under simulated saline conditions.

Hypothesis: There is no significant variability in salt tolerance among *Lathyrus sativus* land races growing in different agro-ecological zone of Tigray. And this null hypothesis was tested at p = 0.05 significant level.
Table 1: Some demographic characteristics of the study areas

<table>
<thead>
<tr>
<th>Name of PA</th>
<th>Endamriam (Dx)</th>
<th>Melfa (Mx)</th>
<th>Agerbea (Ax)</th>
<th>Aregien (Arx)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location</td>
<td>Seret</td>
<td>Melfa</td>
<td>Limat</td>
<td>Aregien</td>
</tr>
<tr>
<td>Population</td>
<td>7,465</td>
<td>6,786</td>
<td>4,798</td>
<td>4,024</td>
</tr>
<tr>
<td>Agro-ecology</td>
<td>Dega</td>
<td>Dega</td>
<td>Dega</td>
<td>Wiene dega</td>
</tr>
</tbody>
</table>

DESCRIPTION OF THE STUDY AREA AND SPECIES

Description of study area: The *Lathyrus sativus* landraces used for this study were collected from Degu’a Tembien district. Degu’a Tembien is one of the 36 administrative districts of Tigray regional state, Northern Ethiopia (Fig. 1). It is bordered by Saharti Samre in the south, Enderta and Wukro districts to the East and North east; Hawezen district to the North; Kola Tembiyen to the West and Tanqua Abergale district to the South West.

Hagereselam (13º30’44” North, 39º11’43” East) is the capital of the district. It is located at altitude of ranging 1500-2750 m.a.s.l. It's well known for its *Lathyrus sativus* cultivation. Agro-ecologically Hagereselam is under the sub humid category having a rainfall of 720 mm with maximum 770 mm and a minimum of 500 mm per annum.

Hagereselam has a temperature range of 11-25ºC which fall under the 'Dega' climate. The soil types predominantly found in Hagereselam are clay (50%), sand (40%) and others cover about 10%. This makes the district predominantly suitable for the growth of grass pea (*Lathyrus sativus*) http://en.wikipedia.org/wiki/Hagere_Selam_(Degua_Tembien).

Geographically, it is located at the Mekelle-Abiyiadi road at a distance of 50 km East of Mekelle, the capital of Tigray. The district has 16 peasant associations under it. Among which 4 of the peasant associations are considered for this study.

Description of the study species: Grass pea (*Lathyrus sativus* L.) is a food, feed and fodder crop belonging to the family Leguminosae (Fabaceae), subfamily papilionoideae), and tribe vicieae. It belongs to genus Lathyrus with a scientific name: of species Lathyrus sativus L. (Cherepanov, 1995). *Lathyrus sativus* is a much branched, struggling or climbing, herbaceous annual, with a well developed taproot system., the rootlets are covered with small, cylindrical, branched nodules, usually clustered together in dense groups.

Experimental methods and design: The study was conducted from December 2010 to June 2011 in the Botanical Sciences Laboratory (BSL) at the department of biology, Mekelle University, Tigray, Northern Ethiopia. Four grass pea (*Lathyrus sativus*) were collected from four different ‘Tabias’ (Peasant associations) of Degu’a Tembien district namely: Endamariam, Melfa, Agerba’a and Aregien (Table 1). Once the different land races were collected and brought to the laboratory, healthy seeds were sorted, surface sterilized with 5% sodium hypochlorite solution for 10 min, rinsed with sterile distilled water several times and placed on Whatman’s Grade filter paper in a Petri dishes. Twenty two seeds per Petri dish were used for the experiment.

All the dishes were arranged in a completely randomized design with with two replicates of each salt treatment. For the salinity test five levels of electrical conductivity designated as: EC<sub>0</sub>, EC<sub>4</sub>, EC<sub>6</sub>, EC<sub>8</sub>, and EC<sub>10</sub> dS/m were used respectively. For this experiment, salinity level was simulated by preparation of with a solution of different concentrations of NaCl and distilled water as control). The design was arranged in such a lay out:

- Land race La + Nacl = 0 ds/m (control)
- Land race La + Nacl = 4 ds/m
- Land race La + Nacl = 6 ds/m
- Land race La + Nacl = 8 ds/m
- Land La + Nacl = 10d ds/m

where, La stands for respectively that were collected from different each peasant associations stated in Table 1. After the 24 h solution preparation 20 petridishes of 9cm were planted with 22 selected *Lathyrus sativus* seeds with two replicates and five factorial design. Seeds were germinated in laboratory at room temperature, the seeds were observed daily and the test solutions were changed on alternate days to avoid salt accumulation. And every day the germinated and emerged seeds were counted. The Percentage of Germination (Pg), the Percentage of Emergence (Pe) and Radical Length (RL) of each landrace was recorded. Furthermore, the seeds water absorption ratio was one of the experimental tests during the lab work and the data was recorded. In addition to that the following data were recorded or/and calculated:

Germination and seedling development:
- Number of days to germinate and germination percentage (%)
- Emergency percentage (%)
- Shoot length (cm) and radical (root) length (cm)

Agronomic and physiological parameters:
- Relative water absorption rate
- Root/shoot ration biomass (Root biomass and shoot biomass).

Seeds were considered to be germinated with the emergence of both shoots and roots as well as shoot branches. The germinating seeds were counted at daily...
Table 2: The effect of different salinity levels on shoot length of lathyrus sativus

<table>
<thead>
<tr>
<th>Landraces</th>
<th>Damariam (DX)</th>
<th>Melfa (Mx)</th>
<th>Agerbe'a (Ax)</th>
<th>Aregien (Arx)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity level</td>
<td>Shoot length</td>
<td>% reduction</td>
<td>Shoot length</td>
<td>% reduction</td>
</tr>
<tr>
<td>4 ds/m</td>
<td>6.15</td>
<td>62.6</td>
<td>8.15</td>
<td>33.2</td>
</tr>
<tr>
<td>6 ds/m</td>
<td>2.82</td>
<td>82.9</td>
<td>2.59</td>
<td>78.8</td>
</tr>
<tr>
<td>8 ds/m</td>
<td>0.00</td>
<td>100</td>
<td>0.48</td>
<td>96.1</td>
</tr>
<tr>
<td>10 ds/m</td>
<td>0.00</td>
<td>100</td>
<td>0.07</td>
<td>99.5</td>
</tr>
</tbody>
</table>

...intervals. Germination percentage, germination index, relative germination rate, relative sodium chloride injury rate, shoot emergency, root emergency percentage and branch emergency percentage of the respective landraces were later calculated using mean of the two replicates by the following formulas of Li (2008) with some modification for the present materials:

- Germination percentage = Total no of seed germinated×100/Total no of seeds taken for germination
- Germination Index (GI) = Total number of germinated seeds /Total no of germination days required
- Relative NaCl injury = Gm % in control- Gm in NaCl treatment /Germination % control

After 20 days of sowing, final germination percentage was calculated, length and fresh weight (g) of shoot and roots were then measured. Finally, tolerance to sodium chloride was determined by calculating sodium chloride response index (SRI) using standard formula: Value from salt treatment×100% for each treatment Value from control

Data analysis: Data obtained from the experiment was organized, analysed and interpreted using JMP 5.0 (the statistical discovery software SAS institute package, 2002) and Microsoft excel software. All data were subjected to statistical analysis of variance (ANOVA) appropriate to the experiment design according to the procedure outlined in SAS package JAMP5. Significance difference between treatments was separated by Duncan’s multiple range test at significance level α = 0.05. A simple regression analysis and graphics were used to show the distribution of the response of land races in and between the agro-ecological zones where the land races were collected from, and between salinity and germination reduction.

RESULTS

Impact of salt on germination: As indicated in Fig. 2 the four land races behaved in similar fashion at lower salinity level (4 and 6 ds/m) except the slight statistically in significant percentage of germination reduction for Aregien (0.2%) at 6 ds/m.

However, at highest salinity levels the land races responded differently. At 8 ds/m the highest percentage of reduction was observed for the landraces from Endamaria (22.7%) and followed by Aregien (13.64%) However, at 10 ds/m germination percentage reduction observed was highest for Aregien (68.2%) and followed by Endamariam (31.2%).

As indicated in Fig. 3. Above, given the highest salinity level, the four landraces showed a statistically significant variation (t-test, t = -0.025, p = 0.0078) in the mean values of, germination percentage, relative germination rate and relative injury rate at highest concentration of 10 ds/m.

Relative NaCl injury rate calculated on the basis of germination percentage in control to the salinity
Impact of salt on seedling growth: Salinity had highly significant effect on shoot length in *Lathyrus sativus*. Shoot length decreased with an increase in salinity level. Maximum reduction 100% of control was observed at 8 dS/m and 10 dS/m for the landrace from Damariam while only at 10 dS/m for the land race from Aregien. At 4 dS/m the reduction observed was 33.2, 41.58, 57.37 and 62.6% of control for Melfa, Aregien, Agerbea and Damariam in that order (Table 2).

Salinity significantly decreased the root length in *Lathyrus sativus* (Table 2 and Fig. 4a). In the control root length was observed 9.305 cm while at 4, 6, 8 and 10 dS/m, respectively it decreased by 43.797 to 86.9% for Endamariam (Table 3 and Fig. 4b). The highest level of root length reduction was observed for the landraces collected from Aregien (100%) followed by Endamariam (86.89%).

The highest shoot dryweight reduction (3%) was observed in the landrace collected from Damariam and Aregien at 6 dS/m. However, the highest biomass reduction (shoot dryweight reduction of 2%) was observed in the landrace collected from Damariam at 4 dS/m followed by Aregien and Agerbea with 1% reduction each (Fig. 5).

**DISCUSSION**

Responses of four different landraces to the five different concentrations of NaCl treatment was tested by mean values of germination percentage, germination index and its relative injury rate during germination and the length of root and shoot emergence was calculated as well. All the landraces indicated a decrease in germination percentage, germination index, germination rate, root emergency percentage and shoot emergency percentage under treatments of 6, 8 and 10 dS/m. Both root growth and shoot growth values of Germination Index (GI) decreased with increasing concentration of NaCl treatment for all land races used in the study. Cumulative effect of NaCl treatment at different concentration on germination percentage was manifested by NaCl induced injury rate. Injury rate was zero (0) at control but increased with increasing concentration of salinity level. This is in line with the findings of Wang et al. (2003). Salt stress declined the germination and also delayed the emergence of seeds in four grass pea landraces. Salt
induced inhibition of seed germination could be attributed to osmotic stress or to specific ion toxicity. It is also assumed that in addition to toxic effects of certain ions, higher concentration of salt reduces the water potential in the medium which hinders water absorption by germinating seeds and thus reduces germination. It appears that a decrease in germination is related to salinity induced disturbance of metabolic process leading to increase in phenolic compounds. It is assumed that germination rate and the final seed germination decrease with the decrease of the water movement into the seeds during imbibitions. Salinity stress can affect seed germination through osmotic effects. Germination percentage also significantly decreased as the level of salinity of the medium increased. These results are similar with the findings of Katerji et al. (1987). However, the results of this finding are different from the reports by Paivoke (2003) who found negative correlation between growth parameters and NaCl treatment but positive correlation with root length and NaCl treatment.

The root and shoot lengths are the most important parameters for salt stress because roots are in direct contact with soil and absorb water from soil and shoot supply it to the rest of the plant. For this reason, root and shoot length provides an important clue to the response of plants to salt stress. High salinity may inhibit root and shoot elongation due to slowing down the water uptake by the plant.

CONCLUSION

To conclude, there is a strong effect on germination of *Lathyrus sativus* with an increase in NaCl concentration. It can be said that salinity effect of NaCl was manifested by significant reduction of different parameters at germination, root and shoot development stage in all land races. Considering the 30.7% reduction of control level in mean value of different parameters: seed germination percentage, relative germination rate and relative sodium chloride injury values of the different traits as determining criteria for sodium chloride tolerance, it was concluded that all the land races of *Lathyrus sativus* are tolerant to NaCl treatment up to 6 dS/m, but become sensitive at higher concentrations during early growth stage. Thus, the information gathered may provide a better strategy in understanding of salinity effect in legumes crops in future germination program.

REFERENCES


