Effects of Na$_2$CO$_3$ on seed germination, seed reserve utilization and seedling growth in bitter vetch (Vicia ervilia L.)

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ABSTRACT

Alkalinity is one of the main limiting factors of seed germination in alkaline soils. The aim of this study was to evaluate the effects of Na$_2$CO$_3$ levels (0, 5, 10, 15, 20 and 25 mM) on seed germination, seed reserve utilization and seedling vigour of three local bitter vetch cultivars (Buinzahra, Shahreza and Tarom). The results indicated that increasing Na$_2$CO$_3$ concentrations caused a decrease in germination percentage (GP), germination rate (GR), seedling length, seedling dry weight (SLDW) and seed reserve utilization efficiency (SRUE). The rate of reduction in seed length in comparison with the control in different concentrations of Na$_2$CO$_3$ was detected from Buinzahra as 30-92%, Shahreza 39-97% and Tarom 13-83%. Decrease in root length was more than shoot length in all the cultivars at each Na$_2$CO$_3$ level, as compared to the control. The weight of mobilized seed reserve (WMSR) and seed reserve depletion percentage (SRDP) decreased with increasing Na$_2$CO$_3$ levels up to 10 mM in Buinzahra and Tarom cultivars and up to 15 mM in Shahreza. WMSR and SRDP then rapidly raised with higher alkalinity levels. These results suggest that reduction of seed germination and decline in seedling dry weight in response to alkalinity stress is a consequence of decline in seed reserve utilization efficiency.

Key words: Alkalinity, Bitter vetch, Germination, Seedling, Seed reserve.

INTRODUCTION

Vicia ervilia L., the bitter vetch, is an ancient important legume crop cultivated extensively in arid regions of Central West Asia and North Africa as high-protein forage for animals with increased grain yield and ability to sustain soil fertility (Samarah et al., 2003; ICARDA, 2004). It has a number of favorable characteristics, such as resistance to drought and insects and good energy and protein content that make it a potential economically useful source for poultry diets. The crop is easy to cultivate and harvest can be grown in very shallow, alkaline soils where other grains such as corn and soybean do not grow successfully (Sadeghi et al. 2009, Enneking and Clive, 1997).

Seed germination is the initial and most crucial stage in the life cycle of plants and determines seedling establishment and plant growth (Zhang et al., 2010). Seed germination is affected by many biotic and abiotic factors, such as temperature, light, water, oxygen concentration, salt and alkalinity. Temperature, salinity, and alkalinity are the main limiting factors in the germination of many species (Lin and Tang, 2005). Alkali salt stress and neutral salt stress are actually two distinct kinds of stresses. Alkali stress is the stress of alkaline salts (NaHCO$_3$ and Na$_2$CO$_3$) and salt stress can be defined as the stress of neutral salts (NaCl and Na$_2$SO$_4$) (Yang et al., 2007). When saline soil contains CO$_3^{2-}$ and/or HCO$_3^-$, it causes injury to plants not only through salt stress, but also through alkali stress (Li et al., 2010a and b; Shi and Wang, 2005). The existence of alkali stress has been demonstrated clearly by a number of studies, which have shown it to be more severe than salt stress (Campbell and Nishio, 2000; Brand et al., 2002). Soil salinity and alkalinity seriously affect about 932 million hectares of land globally, reducing productivity in about 100 million hectares in Asia (Rao et al., 2008). The recent estimates reveal that about 25 million ha of the Iranian lands is saline and/or sodic (SAYYARI and Mahmoodi, 2002).

Soil salinity may affect the germination of seeds either by creating an osmotic potential external to the seed preventing water uptake, or through the toxic effects of ions on the germinating seed (Khajeh-Hosseini et al., 2003). High levels of alkalinity can also be a limiting factor in seed germination (Shi et al., 1998), although this has received less attention in the literature. All the mechanisms of salt tolerance such as excretion, osmotic adjustment using organic solutes and compartmentalization of toxic ions are metabolically energetic and compete with plant growth for resources. Thus, if these mechanisms, elucidated in vegetative plant cells, also operate within the cells of seeds...
germinating in saline environments, it suggests conflicting demands for carbon reserves, both osmotic balance and growth, the balance between which must determine the likelihood of successful germination and seedling growth (Zhang et al., 2010). The response of seed germination to salinity also depends on the availability of stored compounds. High ion content in plant cells can induce changes in storage protein hydrolysis and starch remobilization due to an inhibition of enzyme activity. Therefore, the salt-induced inhibition of seedling growth generally coincides with the delay of reserve mobilization (Voigt et al., 2009).

In the present study, we investigate the effects of alkaline stress on germination and seedling growth of three cultivars of Vicia ervilia L. with emphasis on seed reserve utilization under stress condition and its allocation to the growth.

**MATERIALS AND METHODS**

The experiment was conducted at Seed Research Laboratory of the Department of Agronomy and Plant Breeding, Faculty of Agriculture, Bu-Ali Sina University, Hamedan, Iran. The seeds of V. ervilia were collected from different locations in Iran (Table 1). After collection, the immature seeds and those attacked by insects were removed. The healthy seeds were stored at 5±1 and 50±5% RH until their use.

In order to determine seed water content, three replicates of 50 seeds of each seed lot were weighed (W₁), dried at 104 ±C for 24 h and then reweighed (W₂). Seed water content was calculated as [(W₂−W₁)/W₁]. Treatments consisted of six levels of alkalinity (0, 5, 10, 15, 20 and 25mM Na₂CO₃) with three replications. The Na₂CO₃ concentrations were selected after a preliminary test for tolerance. For each replication, 50 seeds were weighed and the initial seed dry weight was calculated using the data for seed water content. Germination trials were carried out in the dark, in 14-cm petri-dishes containing one disk of Whatman No. 1 filter paper, with 30ml of test solutions maintained in an incubator at 25 ºC. Seeds were surface sterilized with 0.5% sodium hypochlorite solution for 1 min, subsequently washed with distilled water to avoid fungus attack. All Petri dishes and filter papers were also disinfected subsequently washed with distilled water to avoid fungus attack. All Petri dishes and filter papers were also disinfected sterilized with 0.5% sodium hypochlorite solution for 1 min, subsequently washed with distilled water to avoid fungus attack. All Petri dishes and filter papers were also disinfected.

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**RESULTS AND DISCUSSION**

Germination percentage: The results of the ANOVA showed that percentage of germination was affected by the concentration of alkalinity and the interaction between alkalinity and cultivar (Table 2). There was a significant interaction between cultivar and alkalinity levels regarding the germination percentage, indicating that the germination response to Na₂CO₃ concentrations depended on cultivar. The effects of increasing Na₂CO₃ levels on the germination percentage of V. ervilia cultivars are presented in Table 3. In 5 and 10mM levels of Na₂CO₃, the reduction of germination percentage was negligible and there was no significant difference between these concentrations with control treatment. In Buinzahra and Shahreza cultivars, the germination percentage was significantly inhibited when the concentration of Na₂CO₃ was more than 15 mM. The extent

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Life cycle</th>
<th>Harvest site</th>
</tr>
</thead>
<tbody>
<tr>
<td>V. ervilia cv. Buinzahra</td>
<td>Annual</td>
<td>Buinzahra (Qazvin)(Lat:35.77 E , Lon: 50.05 N)</td>
</tr>
<tr>
<td>V. ervilia cv. Shahreza</td>
<td>Annual</td>
<td>Shahreza (Isfahan)(Lat:31.80 E, Lon: 51.89 N)</td>
</tr>
<tr>
<td>V. ervilia cv. Tarom</td>
<td>Annual</td>
<td>Tarom (Zanjan)(Lat:36.73 E, Lon: 48.47 N)</td>
</tr>
</tbody>
</table>
of decrease was more in Buinzahra. The strongest inhibition occurred in the 25 mM alkalinity concentration, particularly in Buinzahra cultivar. Tarom and Shahreza exhibited similar final germination percentages in 20 and 25 mM Na$_2$CO$_3$.

**Germination rate:** Analysis of variance for germination rate indicated that cultivar and alkalinity stress had significant effect for this parameter (Table 2). Germination rate as indicated by the index of germination velocity was much higher for Tarom than for other cultivars (data not shown). Germination rate was progressively inhibited by the increase in alkalinity concentration. The response of seeds to an increase in alkalinity concentration varied among cultivars (Table 4). The decreasing germination rate occurring between 0 and 10 mM Na$_2$CO$_3$ was considered negligible in Buinzahra. Tarom shoot length was more than shoot length in all the cultivars at 20 and 25 mM Na$_2$CO$_3$.

**Table 2:** Analysis of variance for germination percentage (GP), germination rate (GR), seedling length (SLL), weight of mobilized seed reserve (WMSR), seed reserve depletion percentage (SRDP), seed reserve utilization efficiency (SRUE) and seedling dry weight (SLDW)

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>GP</th>
<th>GR</th>
<th>Root Length</th>
<th>Shoot Length</th>
<th>WMSR</th>
<th>SRDP</th>
<th>SRUE</th>
<th>SLDW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivar</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkalinity</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cultivar * Alkalinity</td>
<td>10</td>
<td>129.19**</td>
<td>95.38**</td>
<td>85.61**</td>
<td>218.39**</td>
<td>50.49**</td>
<td>462.11**</td>
<td>0.03**</td>
<td>1.69**</td>
</tr>
</tbody>
</table>

Each result is the average of three replicates. Values followed by the same letter in the table are not significantly different. Different letters indicate significant differences between treatments at the 0.05 level according to LSD test.

**Table 3:** Germination percentage (%) of bitter vetch cultivars in different Na$_2$CO$_3$ levels

<table>
<thead>
<tr>
<th>Alkalinity (mM)</th>
<th>Buinzahra</th>
<th>Shahreza</th>
<th>Tarom</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td>82.81a</td>
<td>78.36b</td>
<td>78.40ab</td>
</tr>
<tr>
<td>5</td>
<td>73.02abc</td>
<td>76.51abc</td>
<td>77.55abc</td>
</tr>
<tr>
<td>10</td>
<td>72.05abc</td>
<td>71.48abc</td>
<td>73.89abc</td>
</tr>
<tr>
<td>15</td>
<td>67.78abc</td>
<td>61.59abc</td>
<td>52.54abc</td>
</tr>
<tr>
<td>20</td>
<td>27.93c</td>
<td>29.65c</td>
<td>39.91cd</td>
</tr>
<tr>
<td>25</td>
<td>14.04c</td>
<td>26.30bc</td>
<td>37.65c</td>
</tr>
</tbody>
</table>

Each result is the average of three replicates. Values followed by the same letter in the table are not significantly different. Different letters indicate significant differences between treatments at the 0.05 level according to LSD test.

**Table 4:** Germination Rate of bitter vetch cultivars in different Na$_2$CO$_3$ levels

<table>
<thead>
<tr>
<th>Alkalinity (mM)</th>
<th>Buinzahra</th>
<th>Shahreza</th>
<th>Tarom</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td>52.02ab</td>
<td>46.24bcd</td>
<td>58.71a</td>
</tr>
<tr>
<td>5</td>
<td>47.55bc</td>
<td>43.62cd</td>
<td>58.43a</td>
</tr>
<tr>
<td>10</td>
<td>47.05bc</td>
<td>34.82ef</td>
<td>47.34bc</td>
</tr>
<tr>
<td>15</td>
<td>39.63f</td>
<td>31.84f</td>
<td>33.16ef</td>
</tr>
<tr>
<td>20</td>
<td>19.84d</td>
<td>12.66b</td>
<td>16.93dh</td>
</tr>
<tr>
<td>25</td>
<td>3.13i</td>
<td>10.16b</td>
<td>16.80ph</td>
</tr>
</tbody>
</table>

Each result is the average of three replicates. Values followed by the same letter in the table are not significantly different. Different letters indicate significant differences between treatments at the 0.05 level according to LSD test.

**Seeding vigour:** The seedling vigour was estimated by means of shoot and root length of seedlings. The effect of cultivar, alkalinity and their interaction for shoot and root length of seedlings was significant (Table 2). Table 5 shows the effect of increasing concentrations of Na$_2$CO$_3$ on the shoot and root length of different cultivars. As expected, increasing alkalinity levels caused remarkably decreases in seedling length (root length + shoot length) of all cultivars. The seedling length of Tarom was significantly shorter than that of Shahreza and Buinzahra at the Na$_2$CO$_3$ concentrations of 0 and 5 mM, while at the concentrations exceeding 5 mM there was no statistically different between the cultivars (data not shown). Among the cultivars Tarom shoot length was affected the least by alkalinity. The rate of reduction in shoot length in comparison with the control in different concentrations of Na$_2$CO$_3$ was detected from Buinzahra as 30-92%, Shahreza 39-97% and Tarom 13-83%. Decrease in root length was more than shoot length in all the cultivars at each Na$_2$CO$_3$ level, as compared to the control.

**WMSR, SRDP, SRUE and SLDW:** Across alkalinity levels, the difference between cultivars was significant for weight of mobilized seed reserve and seed reserve depletion percentage (Table 2). As shown in Fig. 1, cultivar difference for these traits was greater at higher concentrations of Na$_2$CO$_3$ and at lower concentrations (0, 5 and 10 mM), no significant difference among genotypes was observed. WMSR and SRDP decreased with increase in Na$_2$CO$_3$ concentrations up to 10 mM in Buinzahra and Tarom cultivars and up to 15 mM in Shahreza. WMSR and SRDP then rapidly raised with increasing alkalinity level (Fig. 1).
The effect of all sources of variations including cultivar, alkalinity and their interaction was significant for seed reserve utilization efficiency (Table 2 and Fig. 2).

Increasing Na<sub>2</sub>CO<sub>3</sub> levels caused a decrease in seedling dry weight as well as length of seedlings (Table 2 and Fig. 2). There was a remarkable decrease (26 and 21%, respectively) in seedling dry weight under 5 mM Na<sub>2</sub>CO<sub>3</sub> for Buinzahra and Shahreza, but seedling dry weight of Tarom did not show significant difference between the control and 5 mM salinity. Across alkalinity levels lower than 20 mM, seedling dry weight of Buinzahra (initial seed dry weight= 36.67mg) was similar to that of Shahreza (initial seed dry weight= 31.40mg), while in 20 and 25 mM Na<sub>2</sub>CO<sub>3</sub>, Buinzahra exhibited greater performance in respect of

Table 5: Seedling Length (mm) of bitter vetch cultivars in different Na<sub>2</sub>CO<sub>3</sub> levels

<table>
<thead>
<tr>
<th>Alkalinity (mM)</th>
<th>Cultivar</th>
<th>Buinzahra</th>
<th></th>
<th></th>
<th>Shahreza</th>
<th></th>
<th></th>
<th>Tarom</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Root Length</td>
<td>Shoot Length</td>
<td>Root Length</td>
<td>Shoot Length</td>
<td>Root Length</td>
<td>Shoot Length</td>
<td>Root Length</td>
<td>Shoot Length</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>76.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>84.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>65.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27.96&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>42.87&lt;sup&gt;c&lt;/sup&gt;</td>
<td>44.85&lt;sup&gt;b&lt;/sup&gt;</td>
<td>58.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>44.44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24.13&lt;sup&gt;cde&lt;/sup&gt;</td>
<td></td>
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<td></td>
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<tr>
<td>10</td>
<td>21.38&lt;sup&gt;d&lt;/sup&gt;</td>
<td>26.44&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>25.24&lt;sup&gt;d&lt;/sup&gt;</td>
<td>28.71&lt;sup&gt;e&lt;/sup&gt;</td>
<td>28.28&lt;sup&gt;d&lt;/sup&gt;</td>
<td>19.59&lt;sup&gt;de&lt;/sup&gt;</td>
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<tr>
<td>15</td>
<td>14.97&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>18.29&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>11.36&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>20.31&lt;sup&gt;def&lt;/sup&gt;</td>
<td>10.86&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>10.83&lt;sup&gt;ef&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>20</td>
<td>5.04&lt;sup&gt;f&lt;/sup&gt;</td>
<td>14.61&lt;sup&gt;fg&lt;/sup&gt;</td>
<td>5.55&lt;sup&gt;f&lt;/sup&gt;</td>
<td>9.50&lt;sup&gt;gh&lt;/sup&gt;</td>
<td>6.53&lt;sup&gt;fg&lt;/sup&gt;</td>
<td>9.35&lt;sup&gt;gh&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>25</td>
<td>4.44&lt;sup&gt;fg&lt;/sup&gt;</td>
<td>5.03&lt;sup&gt;hi&lt;/sup&gt;</td>
<td>3.23&lt;sup&gt;g&lt;/sup&gt;</td>
<td>1.83&lt;sup&gt;hi&lt;/sup&gt;</td>
<td>4.77&lt;sup&gt;fg&lt;/sup&gt;</td>
<td>4.66&lt;sup&gt;hi&lt;/sup&gt;</td>
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</tbody>
</table>

Each result is the average of three replicates. Values followed by the same letter in the table are not significantly different. Different letters indicate significant differences between treatments at the 0.05 level according to LSD test.
seedling dry weight. The rate of decline in seedling dry weight was more marked in Buinzahra and Shahreza, and Trom was affected the least by increasing alkalinity levels because it gave the lowest reduction rate for SLDW. The decline in seedling dry weight between the control and final alkalinity level (25 mM Na2CO3) was the lowest in Buinzahra and Taram with 75% while the highest reduction was determined on Shahreza with 92%.

The present study indicated that alkalinity has profound effects on seed germination and seed reserve utilization of bitter vetch cultivars. Inhibition of germination due to salt stress has been reported in Kochia prostrata and Kochia scoparia (Orlovsky et al., 2011), Safflower (Kaya et al., 2003), Coriander (Zhang and Zhao, 2011), and Medicago ruthenica (Guan et al., 2009). The decreasing germination due to increasing salinity or alkalinity can be correlated to the nature of the stress to reduce imbibition of water due to lowered osmotic potentials of the medium and causes changes in metabolic activity. Moreover, salinity perturbs plant hormone balance and reduces the utilization of seed reserves (Abdul Jaleel et al., 2007). The effect of CO3 provides the pH effect is also a limiting factor for seed germination (Campbell and Nishio, 2000).

The reduction in germination rate as a result of salt stress has been reported by Ajmal Khan et al. (2001) and Zhanwu et al. (2011). Environmental water potential which surrounds the seed has a direct effect on water absorption rate by seed and existing salts solved in water solution have a notable effect in this process as well.

Increasing alkalinity levels caused remarkably decreases in seedling length of all cultivars. Similar findings have been reported by Shi and Sheng (2005), Guo et al. (2010) and Li et al. (2010) for negative effect of alkaline salt stress on seedling growth. This may be explained by the impact of alkaline stress on root cell development, preventing the uptake of sufficient nutrients, as well as detrimental effects on photosynthesis, which is necessary for biomass accumulation and therefore shoot and root elongation (Walters and Reich, 2000; Paz and Martinez-Ramos, 2003). Furthermore, it is suggested that depression in seedling length under saline stress is attributed to decrease water uptake followed by limited hydrolysis of food reserves from storage tissues as well as due to impaired translocation of food reserves from storage tissue to developing embryo axis (Ghoulam et al., 2002; Lacerda et al., 2003).

Root was found to be more sensitive towards alkalinity than that of shoot, in agreement with Misra and Dwivedi (2004) who reported that radicle was more sensitive to NaCl than plumule during germination of green gram (Phaseolus aureus).

The increase of WMSR and SRDP in high concentrations of Na2CO3 is inconsistent with report of Soltani et al. (2006) who reported a steady decrease in WMSR and SRDP under different salinity and drought levels. It may be due to the increased need for energy and respiration under high alkalinity stress.

Soltani et al. (2001) reported significant cultivar differences for SRUE in wheat while in another study Soltani et al. (2006) showed that the cultivar differences was not significant. The negative effect of alkalinity on seed reserve utilization efficiency in this study is in agreement with findings of Gholami et al. (2009) in Pinto bean (Phaseolus vulgaris L.) and Gholami et al. (2010) in five crop species. Soltani et al. (2002) reported that in chickpea (Cicer arietinum L.) seed reserve utilization efficiency is decreased only at severe salinities, greater than 0.9 Mpa.

CONCLUSION
Successful establishment of plants largely depends on successful seed germination. In this study, under high alkalinity stress (20 and 25 mM Na2CO3), Tarom and Shahreza cultivars had a grater germination percentage than Buinzahra cultivar. Higher germination percentage in these cultivars may be due to the greater mobilized seed reserve and energy expending.

Overall, it seems that decline in seedling dry weight in response to alkalinity stress is a consequence of decline in seed reserve utilization efficiency, which limits the amount of energy available to the seedling during heterotrophic growth. Decrease in SRUE due to salt stress may be a consequence of increased allocation of resources to respiration and salt tolerance mechanisms, which should be investigated in future studies.

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