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Department of Agronomy and Agricultural Extension, University of Rajshahi, Rajshahi 6205, Bangladesh.

Corresponding author & address: Dr. Md. Toufiq Iqbal, E-mail: toufiq_iqbal@yahoo.com

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ABSTRACT


Soil pH regulates poor growth response on wheat seedlings. An open-air plant growth experiment was conducted to determine soil pH effect on growth response to Al-tolerant (ET8) and Al-sensitive (ES8) wheat seedlings. Three different types of soil with different pH’s like 5.3, 8.3 and 8.7 were used in this study. Both ET8 and ES8 were near-isogenic wheat lines that differ by ALMT1 gene were compared. This study showed that seedlings emergence and root proliferation increase with the increase of soil pH. Result also showed that two wheat genotypes responded differently irrespective to soil pH. The ET8 seedlings responded better than the ES8 seedling at all pH level. This study suggested that soil pH also involved in Al tolerance mechanisms in addition to soil available extractable Al. This may be due to ALMT1 gene, which is involved in Al tolerance through malate exudation.

Key words: Al tolerance mechanisms, ALMT1 gene, malate exudation, H⁺-ATPase activity

INTRODUCTION

Soil pH is one of the factor plant growth response (Bose et al. 2010). Generally, low pH soil reduces plant growth and therefore it is necessary to study effect of plant growth in low pH soil (Lazof and Holland, 1999). It is also important to know how plants respond under high H⁺ activities, in order to gain a deeper understanding of Al-tolerance mechanisms. Although some research has examined plant growth response under Al³⁺ activities, few experiments have been conducted under low and high H⁺ activities in growth response to Al tolerant and Al-sensitive wheat seedlings (Ma et al. 2003).

Plant species and genotypes respond differently to the spectrum of soil pH. One solution culture study confirmed that ES8 seedlings grew better at pH 5.5, whereas ET8 seedlings grew better at pH 4.2 (Babourina et al. 2006). This different growth response between ES8 and ET8 may be due to pH differences in the solution (Stewart and Lieffers, 1994). The better growth response of ET8 than that of ES8 at spectrum of pH may also be associated with the loosening of pectin bonds in acidified medium (Cleland 2002). However, it is unclear how these two genotypes behave in both low and high pH soils. Therefore, genotypic variation irrespective to spectrum of soil pH will be considered in this study.

Most of the studies on H⁺ activities have been conducted in nutrient solutions and rooting media which are substantially different from the soil. The primary H⁺ activities in soil-grown plants are not fully understood. Existing solution culture experiments may not be appropriate to understand soil pH activities in soil-plant system. The aim of this experiment is to determine seedlings growth response under different spectrum of soil pH. It was hypothesized that both genotypes will be responded better in high pH soil as compared to low pH soil. It was also hypothesized that the ET8 seedlings will be responded better than the ES8 seedlings.

MATERIALS AND METHODS

Soils

Three types of soils were used in this study. The basic properties of the soils are outlined in Table 1.

<table>
<thead>
<tr>
<th>Soil pH</th>
<th>N (%)</th>
<th>P (ppm)</th>
<th>K (Cmol/Kg)</th>
<th>S (ppm)</th>
<th>Zn (ppm)</th>
<th>Ca (meq/100g)</th>
<th>Mg (meq/100g)</th>
<th>Fe (ppm)</th>
<th>Organic matter (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.3</td>
<td>0.04</td>
<td>9.3</td>
<td>0.15</td>
<td>6.8</td>
<td>0.67</td>
<td>5.10</td>
<td>3.48</td>
<td>18.7</td>
<td>0.52</td>
</tr>
<tr>
<td>5.3</td>
<td>0.04</td>
<td>7.3</td>
<td>0.12</td>
<td>5.1</td>
<td>0.51</td>
<td>2.60</td>
<td>2.16</td>
<td>74.2</td>
<td>0.52</td>
</tr>
<tr>
<td>8.7</td>
<td>0.02</td>
<td>14.8</td>
<td>0.21</td>
<td>1.0</td>
<td>0.61</td>
<td>11.87</td>
<td>2.27</td>
<td>9.6</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Plant materials

Al-tolerant (ET8) and Al-sensitive (ES8) wheat (Triticum aestivum L.) genotypes were used in most of the experiments. These genotypes were near-isogenic (over 95%) lines differing in Al tolerance at the ALMT1 locus (Ahn and Matsumoto, 2006). ET8 and ES8 lines were derived from a cross between the Al-tolerant cultivar Carazinho and the Al-sensitive cultivar Egret, with the resulting progeny backcrossed eight times to Egret or derivatives of Egret (Fisher and Scott, 1987).
Experimental design

The experiment consisted of three kinds of soil pH and two wheat genotypes with three replications. Basal nutrients were not applied to minimize the interactions between soil pH and genotypes, so plant growth over the 13 day study relied on the seed reserve.

Seed germination and plant sowing

Identical size of seeds was selected for germination. The seeds were germinated on Petri dishes with enough moisture. Six holes (1.0 cm depth) were made in each plastic cups containing 200 g soil before sowing. Six pre-germinated uniform seeds of ES8 and ET8 were sown separately in each cup for the three replicates. The germinated seeds were sown in the same way in which radical pointing down and gently cover the seed by same treated soil within the cup. After sowing, each cup was covered by filter paper for first two days to avoid disturbance of top soil and deionized (DI) water was sprayed from top on the filter paper.

Experimental procedure

Six pre-germinated uniform seeds were sown in each replication in plastic cups containing 200 g soil. Soil water content was maintained to 70% field capacity (9% w/w) by weighing each cup. Water was added every day by spraying according to the required volume in each cup separately that was calculated from the weight of each cup including plant and soil. Plants were grown in open air with day/night temperatures variations are 20-30/15-20°C, 10 hr dark and 14 hr light conditions.

Post harvesting activities

Plants were harvested after 13 days of sowing. Plants were not thinned and six plants were considered during each measurement. Whole plants with surrounding root and soil was removed from each cup by gentle agitating to provide minimum disturbance to the roots and shoots. Intact plants were then lifted gently from the soil and shaken lightly to remove bulk soil from the roots. Shoot and root were separated and shoot was stored at 70°C in an oven for minimum 3 days before weight. Harvested roots were washed three times by DI water to remove adhered soil from external root surface. Shoot and root dry weights were weighed after drying.

Statistical analysis

Results were analysed by a two-way analysis of variance (ANOVA) using Genstat 5th ed for Windows (Lawes Agricultural Trust, UK).

RESULTS

Effect of soil pH on emergence of wheat seedlings

Soil acidity reduces the emergence of wheat seedlings. It was found that the highest seedlings emerge occurred in the pH of 8.7. On the other hand, the lowest seedlings emerge occurred in the pH of 5.3 (Fig. 1). Seedlings emergence significantly (P ≤ 0.01) differed under different soil pH. Seedlings emergence tended to be higher in ET8 seedlings than that of the ES8 seedlings (Table 2).
Table 2. Significance levels for the main and interactive effect of soil pH and genotypes on seedlings growth

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>No of plant emergence</th>
<th>Plant height</th>
<th>Shoot dry weight</th>
<th>Root dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil pH</td>
<td>**</td>
<td>**</td>
<td>n.s.</td>
<td>**</td>
</tr>
<tr>
<td>Genotype</td>
<td>n.s.</td>
<td>n.s.</td>
<td>**</td>
<td>n.s.</td>
</tr>
<tr>
<td>Soil pH × Genotype</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Where n.s. and ** represent probability of > 0.05 and ≤ 0.01, respectively. Values were means of three replicates. Plants were harvested after 13 days of sowing.

**Effect of soil pH on plant height of wheat seedlings**

![Plant Height](image1)

Plant height affected by soil pH. The highest plant height of wheat seedlings was found in the pH 8.7. On the other hand, the lowest plant height was obtained in the soil pH 5.3 (Fig. 2). Soil pH and genotype interaction did not significantly (P > 0.05) differ. Likewise, high pH soil did not differ for plant height (Table 2). The ET8 seedlings had higher pH than the ES8 seedlings (Fig. 2).

**Effect of soil pH on shoot dry weight of wheat seedlings**

![Shoot Dry Weight](image2)

The biomass of the seedlings did not differ (P > 0.05) among different soil pH (Table 2). The ET8 seedlings had tended to higher shoot biomass than the ES8 seedlings (Fig. 3).
Effect of soil pH on root dry weight of wheat seedlings

Fig. 4. Root dry weight under different soil pH. Vertical bar represents LSD (P = 0.05) for soil pH × genotype interaction

Root dry weight varied in different soil pH. The highest root dry weight was obtained in the soil pH of 8.7 and the lowest root dry weight was obtained in the pH of 5.3. The ET8 seedlings had tended to higher root dry weight than the ES8 seedlings (Fig. 4; Table 2).

DISCUSSION

Effect of soil pH

Seedlings biomass was lower in low soil pH than high soil pH (Figures 1 to 4). This could be due to availability of H⁺ in the soil solution. The pH increases as the H⁺ concentration declines (Edmeades et al. 1990). As pH increases, Al³⁺ ions sequentially dissociate, releasing OH⁻ ions in place of OH₂ groups, resulting in the formation of increasing insoluble monomers AlOH²⁺, Al(OH)₂⁺ and Al(OH)₃. This results in the reduction of CaCl₂ extractable Al. The decline in the concentration of Al³⁺ means that the soil should become less H⁺ toxic for the wheat seedlings.

Genotypic variations

The ET8 seedlings produced greater plant biomass than ES8 seedlings in both low and high pH (Figures 1 to 4). Many studies explain why ET8 is more Al-tolerant than ES8. Zhang et al. (2001) suggested that Al³⁺ dependent efflux of malate from root apices is a mechanism for Al-tolerance in ET8. The malate anions protect the sensitive root tips by chelating the toxic Al³⁺ cations in the rhizosphere to form non-toxic complexes. Their findings also provided evidence that the higher Al³⁺-induced malate efflux in ET8 than ES8 due to the activation of both malate-permeable and cation channels for sustained malate release. Later, Ahn et al. (2004) reported that the ET8 had higher H⁻-ATPase activities in the plasma membrane resulting in increased transport of H⁻ through the plasma membrane in ET8 compared with ES8. This higher H⁻-ATPase activity and associated increase in H⁺ transport through the plasma membrane in ET8 is also thought to contribute to the difference in Al-tolerance. Thus, the higher malate exudation from ET8 seedlings (Delhaize et al. 1993) via malate-permeable channels is accompanied by the increased zeta potential of the plasma membrane from enhanced H⁻-ATPase activity in ET8, compared with ES8. This indicates that the ALMT1 locus, which was identified as being responsible for the difference in Al-tolerance between ES8 and ET8 (Delhaize et al. 1993) is potentially pleiotropic, having multiple effects from this single gene locus. This was also confirmed by Wherrett et al. (2005), and their findings suggested that the Alh locus may control more than the malate channels in the plasma membrane of ET8. They also suggested that the ET8 had higher Al-induced signalling capacity in its root vacuoles than the ES8, and this also contributed to the greater Al-tolerance in ET8. This proposed mechanism for ET8 was that the Al³⁺ induced the opening of slow Al channels into the vacuole, enabling Al to be sequestered in the root vacuole. Thus, these are a range of proposed mechanisms that contribute to the differential response between ES8 and ET8 that may help ET8, and assist the Al-tolerance in ET8.
CONCLUSION

Soil pH was responsible for the growth response of the wheat seedlings. The increasing level of soil pH enhanced wheat seedlings height, shoots and roots biomass in both genotypes. The ES8 seedlings were more sensitive to H⁺ toxicity than the ET8 seedlings. Different mechanisms were involved in the sensitivity to H⁺ toxicity by the ES8 seedlings. The ES8 seedlings do not secrete malate from its root tips in the rhizosphere. Therefore, they were less able to detoxify Al in the rhizosphere through exudation of malate compared to ET8 as there is possibility to chelate H⁺ in the rhizosphere. Further study will be conducted to examine yield effect of these wheat genotypes under spectrum of soil pH.

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REFERENCES


