Effect of salt and PEG-induced drought stress on germination of sweet sorghum varieties

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Abstract

The effect of salt and drought stress (induced by PEG) on seed germination were investigated in two sweet sorghum (Sorghum bicolor (L.) Moench) varieties namely CSH16 and CSH 30. Five water potentials of the imbibitions solution (from 0 to -1.0 MPa) in NaCl or polyethylene glycol (PEG) for salt and water stress tests, respectively, were studied. Radicle and shoot lengths and dry weights (DWs) were measured 2 days after initial germination. Salt stress decreased FGP, GE % and led to a reduction in shoot and root length and dry weight in both varieties. It was found that the magnitude of reduction, increased with increasing salt stress concentrations. Germination was highly inhibited in both varieties at the 200mM NaCl concentration. Sorghum variety CSH 30 showed greater PEG-induced drought tolerance during germination. The result suggested CSH 30 might be used for further study of drought stress on growth processes and its physiological consequences at an advanced stage of growth.

Keywords: Germination, seedlings, sorghum, salt stress, drought and PEG.

Introduction

Worldwide agricultural productivity has been threatened due to various biotic and abiotic stresses such as drought, salinity, heavy metals and high temperature. Soil salinity and drought is detrimental for agriculture due to the inhibition of crop growth (Anami et al., 2015). Under stress conditions, water deficits in the plant tissues develop, along with changes in biochemical and physiological process in the plant and there is an accumulation of soluble solutes around the seeds which increases osmotic pressure. This affects seed germination and causes unsynchronized seedling emergence effecting the plants’ establishment and causing negative effects on the yield (Wu et al., 2011). The plant adapts to the water stress by altering its cellular mechanism and evoking its defense mechanism (Gruere et al., 2009). Ultimately, an excessive uptake of ions results in toxicity in the plant (Almas et al., 2013).

It is known to survive under severe drought and osmotic stress and is able to recover from alleviation from stress. Selection of plants with a better drought tolerance is critical in dry environments. Successful crop establishment in semiarid regions depends on effective seed germination, which is strictly associated with the ability of seeds to germinate under low water availability (Muscolo et al., 2014).

Sorghum originated from Africa and is currently the fifth most important cereal crop in the world and a staple crop for humans and other animals for
food, feed, fodder, fiber, and fuel (Elikana et al., 2015). Cultivation of sweet sorghum [Sorghum bicolor (L.) Moench] is a high yielding species that is considered a potential energy crop either for ethanol production obtained by fermentation of stalk sugar or for second-generation biofuel obtained from the lignocellulosic biomass (Patanae et al., 2013).

The main objective of this study was to evaluate the influence of salt and drought stress on seeds of two different sweet sorghum genotypes and to understand the effect of these on seed germination. This will in turn allow plant breeders to select cultivars which are best suited for developing salt and drought – resistant cultivars.

**Materials and Methods**

The seeds of two different sweet sorghum varieties used in the study were collected from IARI, Delhi, India. The names of the varieties are CSH 16 and CSH 30. Healthy, uniform seeds of all varieties were surface sterilized with 1% sodium hypochlorite for 3 min, 70% ethanol and distilled water and surface dried using Whatman paper. Twenty seeds for each cultivar in each treatment were allowed to germinate on a filter paper in 9 cm diameter Petri dishes.

For salinity stress, salt solutions used for the germination tests were prepared dissolving 0, 50, 100, 150, 200 mM NaCl in double distilled water. For drought stress, each filter paper was moistened with solutions of 0 (distilled water) as a control, or 10, 15, 18 and 21% of PEG (MW 6000) concentration corresponding to final osmotic potentials of -0.30, -0.51, -0.58 and -0.80 MPa, respectively (Sani and Boureima, 2014). Polyethylene glycol 6000 (HiMedia, Mumbai) is of high molecular weight and is inert and non-ionic. PEG 6000 was used as it is small enough to influence the osmotic potential, but cannot be fully absorbed by the plant and is not phytotoxic (Ellis and Roberts, 1981). 10 ml of appropriate solution was applied to each Petri dish. The Petri plates were arranged in a completely randomized design (CRD) with three replicates of each treatment. The germination room temperature was maintained at 25 ± 1°C in the dark with a 8 h photoperiod and a relative humidity of 70%. Petri plates were periodically checked and respective solutions were applied to compensate evaporation. Seeds were considered germinated when the radicle had extended for at least 2mm. Seedling shoots and root length of randomly selected seedlings from each replication was measured at the time of harvest (4 and 9 days after treatment application) by using a scale. Shoot and root dry weights were recorded after oven drying at 70°C for 24 hours in a hot air oven. After final count, final germination percent (FGP) and germination energy percentage (GE %) were calculated by the following formulae (Ruan et al., 2002 and Hassan et al., 2013).

\[
\text{Number of germinated seeds at 4 DAS x 100} \\
\frac{\text{GE}(\%)}{\text{Total number of seed tested}}
\]

\[
\text{Number final germinated seeds x 100} \\
\frac{\text{FGP}}{\text{Total number of seed tested}}
\]

Seedling vigour index (V) was computed according to Hassan et al. (2013) as follows:

\[
V = L x FG \\
\text{where} \ L \ \text{is the mean seedling length (total shoot and root length in mm) and} \ FG \ \text{is a final germination percentage (\%)}
\]

This experiment was repeated twice to determine the consistency of the results of various varieties against different levels of salt and PEG concentrations.

Data were analyzed using Analysis of Variance method (ANOVA) and least significant difference (LSD) for individual parameters.
Results and Discussion

The ANOVA results showed that there was an effect of drought on all the characters studied at 100 mM NaCl and 15% PEG concentration (The median value) at p ≥0.01.

Final germination percentage

Salt and drought stress induced by PEG had significant effects on the seed germination of CSH 16. In this variety, seed germination was at its lowest at 200 mM NaCl and 21% PEG (Table -1). Germination percentages were inversely related to level of salt and PEG concentration. However, lower salt (50 mM and 100 mM) and PEG concentrations (10, 15 and 18%) did not have any significant effect on the FGP of the CSH 16 variety. Drought results in poor crop stand due to decreased seed germination. Presumably, the osmotic effect due to drought stress was the main inhibitory factor that reduced germination. In CSH 30, the effect of the drought stress was not as drastic. At 200 mM NaCl, the FGP of CSH 16 was 36% as compared to CSH 30 which was 68%. There was a reduction in the FGP with an increase in salt concentration. At 21% PEG, the FGP of CSH 16 was 68% as compared to CSH 30 which was 100%. The high PEG concentration did not seem to have any effect on the FGP of this particular variety.

Seedling vigour index decreased in both the sweet sorghum varieties with increasing salt concentrations (50-200 mM NaCl). Seedling vigour index was the lowest at 200 mM NaCl (Table - 2).

Table - 1. Effect of salt and PEG stress on final germination percentage (%) of sorghum seed varieties after 7 days growth

<table>
<thead>
<tr>
<th>Variety</th>
<th>NaCl Concentration (Mm)</th>
<th>PEG Concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>CSH 16</td>
<td>100±0.58</td>
<td>64±1.19</td>
</tr>
<tr>
<td>CSH 30</td>
<td>92±0.58</td>
<td>90±1.19</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>CSH 16</td>
<td>86±0.19</td>
<td>78±0.2</td>
</tr>
<tr>
<td>CSH 30</td>
<td>100±0.19</td>
<td>96±0.2</td>
</tr>
</tbody>
</table>

Values represent mean ± standard error

Table - 2. Effect of salt and PEG stress on seedling vigour of sorghum seed varieties after 7 days growth

<table>
<thead>
<tr>
<th>Variety</th>
<th>NaCl Concentration (mM)</th>
<th>PEG Concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>CSH 16</td>
<td>2118±0.2</td>
<td>634±0.2</td>
</tr>
<tr>
<td>CSH 30</td>
<td>1681±0.2</td>
<td>1048±0.2</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>CSH 16</td>
<td>1709.68±0.19</td>
<td>1974.18±0.2</td>
</tr>
<tr>
<td>CSH 30</td>
<td>986±0.19</td>
<td>1977.6±0.2</td>
</tr>
</tbody>
</table>

Values represent mean ± standard error
At 10% PEG concentration, there was an increase in seedling vigour index in both the varieties. From this, it can be concluded that low PEG concentrations has a stimulating effect on the germination process and will inhibit the same at higher concentrations (Jayakumar et al., 2008). At 15 and 18% PEG, there is a gradual decrease in seedling vigour index. However, at the highest concentration (21%), there is an increase in CSH 16 and a decrease in CSH 30.

**Germination energy (GE %)**

Germination energy is defined as a percent of seeds germinating in a given period of time. Germination energy was observed at 4th day after soaking of seed and varietal differences were observed in relation to GE % under salt and drought-induced conditions. A significant decrease in germination energy was seen with increasing levels of salt concentration in both the sweet sorghum cultivars. The decrease was more significant for CSH 16. At 50 mM NaCl, this continued to decrease till the GE % was 20 and 68 for CSH 16 and CSH 30 at 200 mM NaCl (Table - 3). There was significant reduction in germination energy with an increase in PEG concentration for CSH 16 (Table - 3). These results are consistent with the work of Sani and Boureima (2014) who described that high dosages of PEG delays germination processes. However, with increasing PEG concentrations, the decrease was not very significant in CSH 30. The increasing PEG concentrations did not seem to affect the germination energy of CSH 30.

**Plumule length**

Plumule length decreased in both varieties with an increase in salt concentration (Fig.- 1). In the control, the plumule length was 11.29 cm in CSH 16
The decrease in plumule length continues till 200 mM where the plumule length was 1.07 cm in CSH 16 and 1.27 cm in CSH 30 (Fig. 1). In CSH 16, there is a gradual decrease seen in plumule length from 11.77 cm in the control to 4.79 cm at 21% PEG concentration. In CSH 30, plumule length decreases from 5.86 cm in the control to 1.94 cm at 21% PEG concentration. However, at 10% PEG concentration, there is an increase in plumule length to 10.43 cm (CSH 30). This could be an osmo-priming effect as it is a lower PEG concentration. Reduction of seedling height is a common phenomenon of many crop plants grown under high salt and drought conditions (Ruan et al., 2002). When subjected to ANOVA, it was found that the results remained significant (p ≤0.01).

**Radicle Length**

Similarly, radicle length also decreased with increasing salt and PEG concentrations (Fig. 2). In CSH 16, the radicle length significantly decreased from control (9.89 cm) to 0.66 cm at 200mM NaCl. With CSH 30, there was an increase at 50mM NaCl from 4.8 cm to 7.13 cm, and then the radical length decreased, at 200mM NaCl, the length was 0.76 cm. With PEG-induced drought stress, it could be observed that there was an increase in both varieties of 10% PEG. The recorded length was 13.99 cm (CSH 16) and 10.17 cm (CSH 30) at 10% PEG. The radical length continued to...

**Table 5. Effect of salt and PEG on radicle dry weight (g/seed) of sorghum varieties**

<table>
<thead>
<tr>
<th>Variety</th>
<th>NaCl Concentration (mM)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CSH16</td>
<td>0.0334</td>
<td>0.0334</td>
<td>0.0108</td>
<td>0.0095</td>
<td>0.0042</td>
<td></td>
</tr>
<tr>
<td>CSH30</td>
<td>0.0254</td>
<td>0.0942</td>
<td>0.0184</td>
<td>0.0073</td>
<td>0.0045</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PEG Concentration (%)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CSH16</td>
<td>0.0352</td>
<td>0.0920</td>
<td>0.0628</td>
<td>0.0094</td>
<td>0.0224</td>
</tr>
<tr>
<td>CSH30</td>
<td>0.021</td>
<td>0.102</td>
<td>0.054</td>
<td>0.097</td>
<td>0.043</td>
</tr>
</tbody>
</table>

Values represent mean ± standard error

**Fig 1.** Effect of salt and PEG induced stress on plumule length (cm) of different sorghum varieties

and 13.47cm in CSH 30. The decrease in plumule length continues till 200 mM where the plumule length was 1.07 cm in CSH 16 and 1.27 cm in CSH 30 (Fig.-1). In CSH 16, there is a gradual decrease seen in plumule length from 11.77 cm in the control to 4.79 cm at 21% PEG concentration. In CSH 30, plumule length decreases from 5.86 cm in the control to 1.94 cm at 21% PEG concentration. However, at 10% PEG concentration, there is an increase in plumule length to 10.43 cm (CSH 30). This could be an osmo-priming effect as it is a lower PEG concentration. Reduction of seedling height is a common phenomenon of many crop plants grown under high salt and drought conditions (Ruan et al., 2002). When subjected to ANOVA, it was found that the results remained significant (p ≤0.01).
Plumule and radicle dry weight

Plumule and radicle dry weight showed an inverse relationship with salt and PEG concentration (Table - 4). Both parameters showed a significant decrease with increasing salt and PEG concentration. Both the varieties gradually decreased with increasing salt concentrations. However, at a 10% PEG, an increase in plumule dry weight was observed in both varieties. The radicle dry weight decreased with increasing salt and PEG levels as presented in Table - 5. At 200mM NaCl and 21% PEG concentration, minimum radicle dry weight was recorded in both the sweet sorghum varieties.

Conclusion

In this study, the effect of salt and drought stress (induced by PEG) on the germination of two sweet sorghum varieties were determined. Characters such as a final germination percentage, germination energy, seedling height, root length and seedling vigour index were studied. From the data, it can be concluded that low concentrations of PEG (0, 10 and 15%) did not have any significant effect on the germination percentage, however, higher PEG concentrations (18 and 21%) negatively affected germination. The highest PEG concentration of 21% least impacted CSH30 variety. This research can be used by plant breeders to select cultivars which are best suited for developing salt and drought – resistant cultivars and for further studies in this field.

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References


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