Effect Of Wax Substrates And Peg Induced Drought Stress In Blackgram (Vigna mungo L.)

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ABSTRACT
At 60% wax concentration, the general mean for root length was higher in the genotype G 6 (9.85 cm). The general mean for shoot length was higher in the genotype G 14 (15.23 cm). Out of twenty genotypes evaluated nine and six genotypes had recorded significant mean value for root length and shoot length respectively. The genotypes G 5, G 6, G 8, G 12 and G 13 penetrate their roots through wax layer. At 65% and 70% wax concentration, the general mean for root length and shoot length was higher in the genotype G 6. The genotype G 12 and G 13 does not germinate at both the concentrations. The genotypes G 5 and G 6 penetrate their roots in 65% wax concentration but not at 70% wax concentration. Three genotypes, G5, G6 and G8 could be considered as drought tolerant genotypes based on root penetration ability and tolerance against PEG-6000.

INTRODUCTION
Onion (Allium cepa var.aggregatum, 2n =2x= 16) belongs to the family Alliaceae, is an important spice crop cultivated all over the world. It has also been shown to be one of the major sources of dietary flavonoids in many countries, specifically, for its flavonol, quercetin and quercetin derivates (Roldan et al., 2008). Drought is a worldwide problem where a major proportion of agricultural land is affected with varying degrees of drought. Water deficit, extreme temperatures and low atmospheric humidity leads to drought, which is one of the most limiting factor for better plant performance and higher crop yield (Szilgyi, 2003; Hirt and Shinozaki, 2003). Water stress affects almost every developmental stage of the plant. However, damaging effects of this stress was more noted when it coincided with various growth stages such as germination, seedling shoot length, root length and flowering (Rauf, 2008; Khayatnezhad et al., 2010). Root penetration ability (RPA) is an important character of onion against drought stress. Root growth is difficult to study, especially in the field. An advantage of the thin wax-layer technique was first developed by Taylor and Gardner (1960). The thin wax-layer technique has been applied successfully in the identification of onion genotypes capable of penetrating hard soils (Babu et al., 2001). Polyethylene glycol (PEG), a series of polymers that vary from viscous liquids to waxy solids has been used to induce water stress artificially (Larher et al., 1993). PEG induced osmotic stress is found to reduce cell water potential (Govindaraj et al., 2010). An increase in concentration of PEG-6000, resulted a decrease in germination rate, root length, shoot length and seed vigor in certain crop plants (Khodarahmpour, 2011).

MATERIALS AND METHODS
The experiment was carried out in the orchard of the Department of Horticulture, Faculty of Agriculture, Annamalai University, Annamalai nagar, Tamil nadu. The experiment site Annamalai nagar is at 11˚24’ North latitude and 79˚41’ East longitude and at an altitude of ± 5.79 m above the mean sea level. A total of 20 genotypes of diverse origin were used for this study. The details of the genotypes used in this study are given in Table 1.

a) Root penetration ability through wax layers

Materials
- 20 genotypes of onion, listed in Table 1.
• Paraffin wax pellets
• Petroleum jelly
• Wooden frame of 3mm height
• Paper cup

**Preparation of wax layers**
The wax layers with different hardness were prepared by melting paraffin wax pellets with petroleum jelly. Wax concentration of different proportions 25:75, 30:70, 35:65, 50:50, 60:40, 65:35 and 70:30 paraffin wax pellets : petroleum jelly (Dang Quy NHAN et al., 2006) were prepared. The mixture was then poured into a wooden frame of 3mm height and allowed to solidify for few minutes. Then the wax layer was cut into circle shape of 53 mm in diameter so as to sealed the bottom of the paper cup.

**Experimental details**

**Experiment 1**
The base of the paper cup was removed and wax disc was installed into it. The gap between wax disc and the side of the cup were sealed by melted wax using micro pipette. The cup was filled with soil and sand in the ratio of 2:1. The bulbs of 20 genotypes were sown in the cup with concentration of 25%, 30%, 35%, 50% and 60%. Then the cups were placed on the tray which was filled with sand. Irrigation was done in alternate days. The experiment was conducted adopting completely randomized block design, replicated thrice.

**Experiment 2**
The five genotypes namely, Kannivadi local, Thuraiyur local, Unjalur local, Chengam local and Oddanchatram local that penetrate the roots at 60% wax were again subjected to screen at 65% and 70% concentration.

**b) Inducing drought with PEG - 6000**

**Materials**
- Five genotypes of onion namely, Kannivadi local, Thuraiyur local, Unjalur local, Chengam local and Oddanchatram local.
- Polyethylene glycol 6000 (PEG- 6000).
- Distilled water.
- Paper cup.

**Method**
Four levels of drought stress, 0 bar (control), -10 bar, -15 bar and -20 bar (Nayer Mohammadkhani and Reza Heidari, 2008) were used in this experiment. The stress levels were prepared by dissolving 0, 30, and 40, 50 g L$^{-1}$ of PEG-6000 in distilled water to obtain 0, -10, -15 and -20 bar drought levels.

**Experimental details**
The bulbs were sown in paper cups which were filled with soil and sand in the ratio of 2:1. Irrigation was done in 3 days interval with PEG solution of 0, -10, -15 and -20 bars to induce different stress levels.

**Results and Discussion**

**a) Root penetration ability through wax layer**

**Experiment 1**
At 60% wax concentration, the general mean for root length was higher in the genotype G 6 (9.85 cm). Out of twenty genotypes evaluated nine genotypes had recorded significant mean value for root length. The general mean for shoot length was higher in the genotype G 14 (15.23 cm). Out of twenty genotypes evaluated six genotypes had recorded significant mean value for shoot length. The genotypes G 5, G 6, G 8, G 12 and G 13 penetrate their roots through wax layer.

**Experiment 2**
At 65% and 70% wax concentration, the general mean for root length and shoot length was higher in the genotype G 6. The genotype G 6 exceeded significant mean value for root length. The genotype G 6 and G 8 exceeded significant mean value for shoot length. The genotype G 12 and G 13 does not germinate at both the concentrations. The genotype G 8 penetrate their roots in both the wax concentration of 65% and 70%. The genotypes G 5 and G 6 penetrate their roots in 65% wax concentration but not at 70% wax concentration. Similar experiments were conducted by Dang Quy NHAN et al., 2006 in rice, Botwright Acuna et al., 2007 in wheat and Lawrence J. Clark et al., 2008 in rice. Dang Quy NHAN reported that Japonica upland rice variety had greater root penetration rate.

**b) Inducing drought with PEG-6000**
At -20 bar, the genotype G 5 recorded the highest mean value for fresh and dry root weight and dry bulb weight. The genotype G 6 was higher for the characters root length, shoot length and fresh bulb weight. The mean value for fresh shoot weight and dry shoot weight was higher in the genotype G 8. Similar result by Bahrami et al., 2012 in sesame cultivar Borazjan for root length, shoot and root dry weights.
Hence, those three genotypes could be considered as drought tolerant genotypes based on root penetration ability and tolerance against PEG-6000.

Table 1. ROOT PENETRATED GENOTYPES THROUGH WAX LAYER

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Root length (cm)</th>
<th>Shoot length(cm)</th>
<th>Root penetrated genotypes through wax layer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>65%</td>
<td>70%</td>
<td>65%</td>
</tr>
<tr>
<td>G 5</td>
<td>3.78</td>
<td>1.75</td>
<td>3.96</td>
</tr>
<tr>
<td>G 6</td>
<td>6.07**</td>
<td>4.11**</td>
<td>10.54**</td>
</tr>
<tr>
<td>G 8</td>
<td>4.14</td>
<td>0.95</td>
<td>6.31*</td>
</tr>
<tr>
<td>G 12</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>G 13</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>General</td>
<td>2.80</td>
<td>1.36</td>
<td>4.16</td>
</tr>
<tr>
<td>CD (0.05)</td>
<td>1.58</td>
<td>1.51</td>
<td>1.91</td>
</tr>
<tr>
<td>CD (0.01)</td>
<td>2.49</td>
<td>2.37</td>
<td>3.00</td>
</tr>
<tr>
<td>SEd</td>
<td>0.61</td>
<td>0.58</td>
<td>0.74</td>
</tr>
</tbody>
</table>

*Significance at 5 % level  **Significance at 1% level

✓ Root penetrate  X Root not penetrate

Table 6. Selection of genotypes based on drought tolerance

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Root penetrated genotypes through wax layer</th>
<th>Root length (cm)</th>
<th>Dry bulb weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>65%</td>
<td>70%</td>
<td>-20 bar</td>
</tr>
<tr>
<td>G 5</td>
<td>RP</td>
<td>NRP</td>
<td>6.32**</td>
</tr>
<tr>
<td>G 6</td>
<td>RP</td>
<td>NRP</td>
<td>7.48**</td>
</tr>
<tr>
<td>G 8</td>
<td>RP</td>
<td>RP</td>
<td>7.48**</td>
</tr>
</tbody>
</table>

RP-Root penetrated genotypes  **Significance at 1% level
NRP-Non root penetrated genotypes

Plate 1. Wooden Frame Of 3 Mm Height Used To Prepare Wax Discs
PLATE 2. WAX DISCS OF 3mm HEIGHT AND 53 MM DIAMETER
PLATE 3. ROOT PENETRATION ABILITY OF ONION GENOTYPES FOR VARIOUS 60% CONCENTRATION

PLATE 4. ROOT PENETRATION ABILITY OF ONION GENOTYPES FOR VARIOUS 65% CONCENTRATION

PLATE 5. ROOT PENETRATION ABILITY OF ONION GENOTYPES FOR VARIOUS 75% CONCENTRATION
PLATE 6. INFLUENCE OF PEG-6000 ON DROUGHT TOLERANCE

REFERENCES