Seed germination and seedling growth responses of *Plectranthus amboinicus* (Lour.) Spreng. to drought stress induced by polyethylene glycol

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Abstract

Drought is an important factor that negatively affects plant growth. PEG-6000 was used to induce drought stress as it can modify the osmotic potential of nutrient solution cultures. Genus *Plectranthus*, from Lamiaceae family, is famous for its medicinal value and apart from its water conservation mechanism which was under wide cultivation in tropical countries including India. Intending to get insight into the polyethylene glycol induced drought stress responses, the present study focuses to evaluate the seed germination, seedling growth under stressed condition (Shoot length, Root length) and seedling fresh weight response of *Plectranthus amboinicus* (Lour.) Spreng. PEG 6000 applied with elevated concentrations (1, 5, 10, 15, 20, 25%) reduces the seed germination and seedling growth responses of *Plectranthus amboinicus* (Lour.) Spreng, in a concentration-dependent manner. Maximum inhibition of seed germination occurred at the Highest PEG concentration (28.3%), Root length (0.9 cm), shoot length (1.6 cm), seedling fresh weight (2.89 mg), seedling dry weight (0.11 mg) when compared with control values of respective trait as 89.44%, 2.1 cm, 2.5 cm, 8.42 mg and 0.35 mg respectively.

Keywords: *Plectranthus amboinicus*, PEG-6000, drought stress, seedling growth, germination

Introduction

Genus *Plectranthus*, from Lamiaceae family, is famous for its medicinal value and apart from its water conservation mechanism which was under wide cultivation in tropical countries like India. *Plectranthus amboinicus* (Lour.) Spreng. is a medicinal and aromatic perennial herb commonly known as Indian borage in English, patherchur in Hindi and locally named as “Bhambri” is native to Taiwan. Water is essential for normal functioning and metabolism of plants while water deficit has a negative influence on plant growth, development, and production (Wu et al., 2008; Efeoglu et al., 2009) which limits crop production worldwide. Drought is an important factor that negatively affects plant growth which includes a reduction in germination percentage, seedling growth performance and fresh weight of the plant (Wu et al., 2019). However, under different stress conditions, the free radical generation exceeds the overall cellular antioxidative potential leading to oxidative stress, which contributes to adverse effects on plant growth (Sharma and Kumar, 2015) (13). To overcome stress plants, have an efficient defense system comprising a set of enzymatic as well as non-enzymatic antioxidants (Kumar and Thakur, 2019). The research efforts have been focused on understanding the mechanisms driving plant response and adaptation to water deficit in the soil at morphological, physiological, and molecular levels (Rivas-Ubach et al., 2014; Bechtold and Field, 2018) (13, 2). Polyethylene glycol (PEG) is being widely used to induce drought-like stress responses in plants (Skriver and Mundy, 1990). It causes osmotic stress. The physiological responses to soil drought and PEG stress are including inhibition of photosynthesis and photosystem II, lower leaf relative water content, increased osmotic regulation, and enhanced antioxidant capacity (Cui et al., 2018). However, these physiological responses differ depending on the stress time, stress type and stress responses including phytohormonal balance, proline content, and leaf water content. These can change within a few hours under PEG stress, whereas soil drought treatments must last for days or longer because control of soil moisture levels cannot be altered in a short period of time (Budak et al., 2013) (3). Furthermore, the molecular characteristics of plants differ between soil drought and PEG stresses (Forner-Giner et al., 2011) (8).
Plants have evolved multiple strategies to overcome drought stress, including drought escape via developmental plasticity and drought tolerance via enhanced osmotic adjustment, water absorption, antioxidant capacity, and stomatal adjustment (Farooq et al., 2009) [7]. Osmotic regulation is crucial for drought resistance in plants. Several osmotic regulator proteins such as dehydrin, late embryogenesis abundant proteins, aldehyde dehydrogenase, and pyrroline-5-carboxylate synthetase, significantly increased in plants under water deficit (Li et al., 2018) [11]. These proteins play important roles in protecting cells from dehydration stress (Samarah, 2016) [14]. Plants have species-specific variations with regard to the tolerance to diverse abiotic stresses. Certain drought stress-tolerant plant species possess the ability to cope up extreme conditions like drought stress with low toxic influence. Such plants are central to a screening of stress-tolerant plants. Apart from agriculture crops, medicinal plants with a wide range of commercial importance were also victims of drought stress.

For a sustainable agriculture response of a species that need to be understood to abiotic stress conditions like drought stress which was so far not focused on species of this genus. To get insight into the polyethylene glycol induced drought stress responses, the present study focuses to evaluate the seed germination, seedling growth under stressed condition (Shoot length, Root length) and seedling fresh weight response of Plectranthus amboinicus (Lour.) Spreng.

**Material and methods**

**Seed source**

Seeds of Plectranthus amboinicus (Lour.) Spreng. were procured from College of Horticulture and Forestry, Neri, district Hamirpur under Dr. YSP University of Horticulture and Forestry, Solan, Himachal Pradesh.

**Seed germination assay**

Uniform seeds of all Plectranthus amboinicus (Lour.) Spreng. were surface sterilized with 0.1% HgCl₂ for 3 minutes followed by thorough washing with distilled water to remove any traces of sterilizing agent (Deswal, 2012) [5]. They were soaked in solutions (10 ml) of different of polyethylene glycol (1, 5, 10, 15, 20, 25%) in the form of PEG 6000 for 24 hours. Seeds simultaneously soaked in distilled water constituted the control. Thereafter, the seeds were transferred to Petri plates lined with a double layer of Whatman filter paper made wet with 2 ml of respected solutions of Hg concentrations. The Petri dishes were placed in the plant growth chamber at 30 ± 2°C under continuous illumination (PAR: 40 μmol m⁻² s⁻¹) for seed germination and seedling growth. The emergence of a 2 mm radicle was taken as seed germination (ISTA, 1996) [9].

**Seed germination**

Seed germination was recorded After 10-day treatment. The total number of germinated seeds was counted and recorded. Germination percentage was calculated as the Number of seed germinated/ Total number of seed × 100. Germination percentage was also used to calculate vigor index I as: germination percentage × (shoot length +Root length).

**Shoot length**

The seedling growth in terms of shoot length was measured after 10 days of treatment. Shoot length was measured from shoot base up to shoot apex in centimeters with the help of a geometrical scale.

**Root Length**

The seedling growth in terms of root length was measured after 10 days of treatment. Root length was measured from root base up to root apex in centimeters with the help of the geometrical scale.

**Seedling fresh and dry weight**

Seedling fresh and dry weight was measured with the help of electronic weighing balance and recorded in milligrams. Seedling dry weight was used to calculate the Vigor index II as germination percentage × seedlings dry weight.

**Statistical analysis**

All data were analyzed by using one-way analysis of variance (ANOVA). Means of all values were compared by Tukey’s test to detect the difference between treatments. A least significant difference (LSD) was calculated and an ‘F’ test was applied to assess the significance of data at 5% levels of probability (P< 0.05). Standard error was plotted in all the graphics.

**Results and discussion**

**Seed germination:**

Elevated concentrations of PEG 6000 suppressed the seed germination in Plectranthus amboinicus (Lour.) Spreng. After 10 days of incubation highest, PEG 6000 concentration (25%) showed maximum inhibition of 28.33% (Fig. 1). Germination inhibition occurred in a concentration-dependent manner.

![Fig 1: Effect of PEG 6000 on germination of Plectranthus amboinicus (Lour.) Spreng. Data are arithmetic means ± S.E.](image)

Maximum germination was evident in control (89.44 ± 2.9) and then germination percentage was decreased with increasing concentration of PEG 6000. Thus at 10 and 20% PEG 6000 concentration, the germination percentage was 56.33 ± 2.91 and 38.67 ± 2.91 respectively. The finding showed that Plectranthus amboinicus (Lour.) Spreng. plant is sensitive to PEG induced drought stress at least for seedling stages. A similar type of observation was reported by Wu et al., 2019 [18] that PEG 600 induced drought stress in Echinochloa crusgalli decreases the germination percentage with increase PEG concentration. Hydraulic reduction reduces water entrance in seeds and increases the drought stress and influences the rate of germination, metabolic and physiological processes (Masoumi A, et al., 2007) [12]. The type and magnitude of PEG6000 induced seed germination appeared to be determined by the degree of permeability of seed coat to HM ions in different species. PEG uptake by
seeds occurred in this study that is evident from the suppression of seed germination.

Root length
PEG 6000 induced Suppression of root length (Fig 2). the highest inhibition of root length due to PEG 6000 treatment occurred at the highest (25%) concentrations. root growth was inhibited in a concentration depended manner. Root length inhibition at 5, 15 and 25% PEG 6000 concentrations was 1.7 ± 0.18, 1.3 ±0.12 and 0.9 ± 0.15 centimetre respectively. A similar type of observations was reported by Wu et al., 2019 [18] that PEG 600 induced drought stress in Echinochloa crusgalli decreases the root length with increase PEG concentration

![Fig 2: Effect of PEG 6000 on root length of Plectranthus amboinicus (Lour.) Spreng. Data are arithmetic means ± S.E.](image)

Shoot length
Lower concentrations of PEG 6000 are not inhibitory for shoot length while increasing concentration of PEG 6000 decreases the shoot growth. Maximum inhibition of shoot length occurs at 20 and 50% concentrations thus the shoot length at these respective concentrations was 1.7 ± 0.3 and 1.6 ± 0.3 cm respectively (Fig 3). Wu et al., 2019 [18] reported a similar type (decrease in shoot length with an increase in PEG 6000 concentration) of inhibition in shoot length.

![Fig 3: Effect of PEG 6000 on shoot length of Plectranthus amboinicus (Lour.) Spreng. Data are arithmetic means ± S.E.](image)

Seedling fresh weight
Seedling fresh weight decreases with an increased concentration of PEG 6000. Thus at 5, 15 and 25% PEG 6000 concentrations fresh weight was recorded as 6.41 ± 0.21, 5.0 ± 0.16 and 2.89 ±0.23 milligram respectively (Fig 4).

![Fig 4: Effect of PEG 6000 on seedlings fresh weight of Plectranthus amboinicus (Lour.) Spreng. Data are arithmetic means ± S.E.](image)

### Table 1: Seedlings dry weight and vigor index at different concentration of PEG 6000

<table>
<thead>
<tr>
<th>PEG6000 concentrations</th>
<th>Seedling dry weight (mg)</th>
<th>Vigor index I</th>
<th>Vigor index II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.35 ± 0.03</td>
<td>225.7</td>
<td>31.3</td>
</tr>
<tr>
<td>1%</td>
<td>0.34 ± 0.03</td>
<td>148.5</td>
<td>24.9</td>
</tr>
<tr>
<td>5%</td>
<td>0.29 ± 0.04</td>
<td>127.5</td>
<td>19.2</td>
</tr>
<tr>
<td>10%</td>
<td>0.28 ± 0.04</td>
<td>102.6</td>
<td>15.7</td>
</tr>
<tr>
<td>15%</td>
<td>0.26 ± 0.02</td>
<td>82.3</td>
<td>11.7</td>
</tr>
<tr>
<td>20%</td>
<td>0.19 ± 0.02</td>
<td>66.7</td>
<td>7.3</td>
</tr>
<tr>
<td>25%</td>
<td>0.11 ±0.01</td>
<td>46.2</td>
<td>3.1</td>
</tr>
</tbody>
</table>

Seedling dry weight and Vigor index
Seedling dry weight showed concentration-dependent behavior of reduction. Seedling dry weight was recorded highest for control. At 5, 15 and 25% PEG 6000 concentrations the seedling dry weight was 0.29 ±0.04, 0.26 ± 0.02 and 0.11 ± 0.01 respectively (Table 1). Vigor index is the total of those properties of the seed which determine the level of activity and performance of the seed during germination and seedling emergence. Vigor index value decreases with an increase in the concentration of PEG in concentration dependent manner.

Conclusion
In conclusion, our findings revealed PEG 6000 induced drought stress is generally inhibitory for seed germination and seedling growth responses of Plectranthus amboinicus (Lour.) Spreng. at least at the seedling stage. Further drought stress mechanisms could be understood by studying antioxidative properties of different enzymes that are involved in abating ROS and eventually drought stress. However, it is not clear whether the seed germination suppression by Hg was related to differential PEG uptake as we have not measured the PEG contents of seed.

References


