Drought Stress Response in *Clitoria ternatea* L.

Abstract

The drought stress is one of the most important environmental stresses that effect the development and production of the plants. The impact of drought stress on seed germination, root and shoot length and fresh weight was studied in Clitoria ternatea L. (Butterfly Pea) which is a medicinal plant. The drought stress was induced by PEG-6000 (Polyethylene glycol). Different concentrations of PEG-6000 (1%, 5%, 10%, 15%, 20%, 25% and 30%) were used. The goal of this paper was to determine the drought stress induced by PEG 6000 (Polyethylene glycol) on seed germination and morphological changes in the plant (shoot length, root length, and fresh weight).

I. INTRODUCTION

*Clitoria ternatea* L., commonly known as butterfly pea belonging to family fabaceae. *Clitoria ternatea* L. is widely grown as medicinal, fodder and ornamental plant [11]. *Clitoria* L. Comprises 60 species and the most frequently reported species is *Clitoria ternatea* L. *Clitoria ternatea* L. has twining fine stem, leaves are pinnate with 5-7 elliptic to lanceolate leaflets, 3-5 cm long. Flowers are solitary deep blue; very short and pedicellate 4-5 cm long. Pods are flat, linear, beaked, 6-12 cm long, 0.7-1.2 mm wide and slightly pubescent with up to 10 seeds. Seeds are olive brown or black in colour, 4.5-7 mm long and 3-4 mm wide [8]. *Clitoria ternatea* L. is a medicinal plant. The whole plant is used for medicinal purposes. The *Clitoria ternatea* L. plant contains various secondary metabolites like flavonoids, anthocyanin, glycosides, pentacyclic triterpenoids and phytosterols [21]. The roots are useful in asthma, burning sensation, inflammation, leprosy, hemicranias, pulmonary tuberculosis, laxative, diuretic, aphrodisiac, tonic [14].
The roots, stem and flower are recommended for the treatment of snakebite and scorpion sting in India [22]. The plant is used especially for boosting memory and improving intellect [23]. Antioxidant, antidiabetic and hepatoprotective activities also reported in *Clitoria ternatea* L. [15].

Plant growth, development, and production are affected by natural stresses in the form of abiotic and biotic stresses such as drought, salinity, freezing inversely, viruses, bacteria, and fungi. Drought stress is one of the most important environmental stresses and remains an ever-growing problem that severely limits crop production worldwide and causes important agricultural losses particularly in arid and semiarid areas [4]. Drought affects morphological, physiological, biochemical and molecular processes in plants resulting in growth inhibition, stomata closure with consecutive reduction of transpiration, chlorophyll content, and inhibition of photosynthesis and protein changes [19, 27] to cope with osmotic changes in their tissues. Drought stress may damage oxygen-evolving complex of photosystem I and PS II reaction centres [25] due to which photosynthetic rate is affected. Generally, legumes are highly sensitive to water deficit stress [18]. Drought stress is known to increase the number of secondary metabolites in a variety of medicinal plants example artemisinin in leaves of *Artemisia annua* L. [5] and ajmalicine in *Catharanthus roseus* roots [12].

II. MATERIALS AND METHODS

To achieve the objectives, following experiments were conducted to explore the effects of different levels of drought stress on *Clitoria ternatea* L.

2.1. Materials

We obtained Seeds of *Clitoria ternatea* L. from Y.S Parmar University of Horticulture and Forestry, District-Hamirpur, Himachal Pradesh. Experiments were conducted in the laboratory of Department of Bioscience, Division- Botany, Career Point University, Hamirpur (Himachal Pradesh).

2.2. Methods

Homogenous seeds of *Clitoria ternatea* L. were surface sterilized for 3 minutes in 0.1% mercuric chloride and rinsed two times with distilled water to remove all the traces of sterilizing agent [6]. Polyethylene glycol (PEG) solution with molecular mass of 6000 has been used to create an osmotic stress [9]. For a preliminary screening, different concentrations of PEG (1, 5, 10, 15, 20, 25, 30, 35 and 40%) were used. Seed germination percentage was completely inhibited at 30, 35 and 40% PEG concentration. A substantial change in seed germination percentage and morphological traits of seedling was recorded as 1, 5, 10, 15, 20 and 25% PEG. Therefore, these concentrations of PEG were selected for the experiment. Seeds were soaked in distilled water (Control) or in six different concentrations of PEG (1, 5, 10, 15, 20 and 25%) for 12h at 25±2°C. Ten replicates of 10 seeds were placed in sterile Petri dishes lined with a two filter paper with 5ml of distilled water or the respective PEG solutions. Seeds were allowed to germinate under natural conditions in a laboratory at 30°C (day temperature) and 20°C (night
temperature) for 15 days. Seeds of *Clitoria ternatea* L. started germinating within a day. A seed was considered as germinated when the emerging radical elongated to 2 mm [2, 10]. After 15 days the numbers of germinated seeds were counted and morphological traits such as root length shoot length and seedlings of fresh weight were measured.

### III. RESULTS AND DISCUSSION

#### 3.1. Seed Germination

Seed germination was significantly affected by drought stress in *Clitoria ternatea* L. (Table 1 and Fig 1). The seed germination percentage decreased with increase in the concentration of PEG but increased at moderate (10%) and severe (20%) PEG concentration. Germination percentage at control, 1% PEG, 5% PEG, 10% PEG, 15% PEG, 20% PEG and 25% PEG was 40%, 27.5%, 32.5%, 42.5%, 27.5%, 42.5% and 17.5% respectively. After 15 days of drought stress germination percentage decreased significantly (P < 0.05) from 40% in control to 17.5% in 25% PEG concentration, whereas it increased non significantly (P > 0.05) by 42.5% in 10% and 20% PEG concentration with respect to control. Increased germination percentage in the *Clitoria ternatea* L. indicates that the plant is tolerant to drought at moderate and severe stress conditions. PEG treatments decrease water potential gradient between seeds and their surrounding media and resulted in reduction in germination percentage. Our results are in agreement with the findings of Gupta *et al.*, (1993); Kaur *et al.*, (2000), Zeid and Shedeed, (2006), and Amin *et al.*, (2011), who revealed that PEG induced drought stress resulted in reduced seed germination in *Cicer arietinum, Medicago sativa*, and okra plant, respectively.

![Figure 1](URL): Effect of different levels of drought stress on Seed germination of *Clitoria ternatea* L., (± S.E; n= 4).
Table 1: Effect of different levels of drought stress on seedling growth in *Clitoria ternatea* L., after 15 days of incubation. Each value is mean ± S.E. of four replicates.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Treatment</th>
<th>Germination (%)</th>
<th>Shoot Length (cm)</th>
<th>Root Length (cm)</th>
<th>Fresh weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control</td>
<td>40±7.9</td>
<td>6.4±0.3</td>
<td>2.5±0.1</td>
<td>260±12.2</td>
</tr>
<tr>
<td>2.</td>
<td>1% PEG</td>
<td>27.5±4.1</td>
<td>5.6±0.4</td>
<td>1.6±0.1</td>
<td>240±15.2</td>
</tr>
<tr>
<td>3.</td>
<td>5% PEG</td>
<td>32.5±4.1</td>
<td>4.9±0.3</td>
<td>1.6±0.2</td>
<td>230±9.35</td>
</tr>
<tr>
<td>4.</td>
<td>10% PEG</td>
<td>42.5±7.3</td>
<td>6±0.1</td>
<td>1.5±0.2</td>
<td>240±9.35</td>
</tr>
<tr>
<td>5.</td>
<td>15% PEG</td>
<td>27.5±8.9</td>
<td>5.3±0.2</td>
<td>1.8±0.2</td>
<td>240±13.9</td>
</tr>
<tr>
<td>6.</td>
<td>20% PEG</td>
<td>42.5±9.6</td>
<td>5.9±0.1</td>
<td>1.9±0.1</td>
<td>250±12.2</td>
</tr>
<tr>
<td>7.</td>
<td>25% PEG</td>
<td>17.5±2.1</td>
<td>6.5±0.3</td>
<td>1.7±0.3</td>
<td>190±7.9</td>
</tr>
</tbody>
</table>

Figure 2: Effect of different levels of drought stress on seed germination of *Clitoria ternatea* L., after 15 days of incubation.

Figure 3: Effect of different levels of drought stress on seedling growth in *Clitoria ternatea* L., after 15 days of incubation.
3.2. Seedling Shoot Length

Seedling shoot length was significantly affected by drought stress (Table 1 and Fig 3) Elevated PEG concentration decreased the shoot length except at 25% PEG concentration. After 15 days of drought stress seedling shoot length observed in control, 1% PEG, 5% PEG, 10% PEG, 15% PEG, 20% PEG and 25% was 6.4 cm, 5.6 cm, 4.9 cm, 6 cm, 5.3 cm, 5.9 cm and 6.5 cm, respectively (Fig 4). The minimum (23.43%) and maximum (1.56%) seedling shoot length measured at 5% PEG concentration, compared with control (P < 0.05) and 25% PEG concentration, compared with control (P > 0.05). Our results are in agreement with the findings of Specht et al., (2001) and Kalarovic et al., (2006) who revealed that PEG-induced drought stress resulted in reduced shoot length in soybean and maize, respectively. The shoot length was decreased under drought stress because it absorbed less water that means the plant is drought sensitive.

3.3. Seedling Root Length

The increase in the concentration of drought stress is accompanied by a decrease in the seedling root length (Table 1 and Fig 5). After 15 days of drought stress seedling shoot length observed in control, 1% PEG, 5% PEG, 10% PEG, 15% PEG, 20% PEG and 25% was 2.5 cm, 1.6 cm, 1.6 cm, 1.5 cm, 1.8 cm, 1.9 cm and 1.7 cm, respectively (Fig 5). The minimum (1.5 cm) and maximum (1.9 cm) seedling root length measured at 10% PEG concentration, compared with control (P < 0.05) and 20% PEG concentration, compared with control (P > 0.05). It decreased significantly (P < 0.05) by 40% and increased non-significantly (P > 0.05) by 2.4% in comparison to control. Our results are in agreement with the findings of Kalarovic et al., (2006) and Blum et al., (1998), who revealed that PEG induced drought stress resulted in reduced root length in maize and wheat, respectively. Root length was decreased under drought stress because it absorbed less water that means the plant is drought sensitive.
Figure 4: Effect of different levels of drought stress on (A) Shoot length, (B) Root Length and (C) Fresh weight of seedling of *Clitoria ternatea* L., (± S.E; n= 4).

3.4. Seedling Fresh Weight
Drought stress imposed by PEG significantly decreased the seedling fresh weight (Table 1 and Fig 6). After 15 days of drought stress seedling shoot length observed in control, 1% PEG, 5% PEG, 10% PEG, 15% PEG, 20% PEG and 25% was 260 mg, 240 mg, 230 mg, 240 mg, 250 mg and 190 mg, respectively (Fig 6). The minimum (190 mg) and maximum (250 mg) seedling fresh weight measured at 25% PEG concentration, compared with control (P < 0.05) and 20% PEG concentrations, compared with control (P > 0.05). It decreased significantly (P < 0.05) by 11.53% and increased non-significantly (P > 0.05) by 3.84% in comparison to control. Our results are in agreement with the findings of Michalek and Browski (2005) and Kerepesi and Galiba (2000), who revealed that PEG induced drought stress resulted in reduced fresh weight in soyabean and wheat respectively. Fresh weight was decreased under drought stress because seedling of the plant absorbed less water that means the plant is drought sensitive.

IV. CONCLUSION
The present study on effect of different levels of drought stress on morphological characteristics of seedling of *Clitoria ternatea* L. over period of time had revealed the following important observations:
1. Minimum germination percentage (17.5%) was observed at severe level of drought stress that is 25% PEG in comparison to control (40%). It decreased significantly by 56.25% in comparison to control (P < 0.05). Maximum germination percentage (42.5%) was observed at 10% and 20%PEG. It increased non-significantly by 6.25% in comparison to control (P > 0.05).
2. Minimum shoot length (4.9 cm) was observed in 5%PEG in comparison to control (6.4 cm). It decreased significantly by 23.43% in comparison to control (P < 0.05). Maximum
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Shoot length (6.5 cm) was observed in 25% PEG. It increased non-significantly by 1.56% in comparison to control (P > 0.05).

3. Minimum root length (1.5 cm) was observed in 10% PEG. It decreased significantly by 40% in comparison to control (P < 0.05). Maximum root length (1.9 cm) was observed in 20%. It increased non-significantly by 2.4% in comparison to control (P > 0.05).

4. Minimum fresh weight (190 mg) was observed in 25% PEG in comparison to control that is 260 mg. It decreased significantly by 11.53% in comparison to control (P < 0.05). Maximum fresh weight (250 mg) was observed in 20% PEG Concentration. It increased non-significantly by 3.84% in comparison to control (P > 0.05).

From the present study it can be concluded that drought stress reduced plant growth. Results of present study revealed that reduction in germination percentage, shoot length, root length and fresh weight in comparison to control was observed. Therefore, the Clitoria ternatea L. can be considered as drought sensitive plant.

V. REFERENCES


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