EFFECT OF POLYETHYLENE GLYCOL INDUCED DROUGHT STRESS ON PHYSIO-HORMONAL ATTRIBUTES OF SOYBEAN

MUHAMMAD HAMAYUN1, SUMERA AFZAL KHAN2, ZABTA KHAN SHINWARI3, ABDUL LATIF KHAN1,4, NADEEM AHMAD1 AND IN-JUNG LEE 1*

1School of Applied Biosciences, College of Agriculture and Life Sciences, Kyungpook National University, Korea
2Center of Biotechnology and Microbiology, University of Peshawar, Pakistan
3Department of Plant Science, Quaid-i-Azam University, Islamabad, Pakistan
4Kohat University of Science & Technology, Kohat Pakistan

Abstract

Drought stress is a major abiotic constraint limiting crop production world wide. In current study, we investigated the adverse effects of drought stress on growth, yield and endogenous phytohormones of soybean. Polyethylene glycol (PEG) solutions of elevated strength (8% & 16%) were used for drought stress induction. Drought stress period span for two weeks each at pre and post flowering growth stage. It was observed that soybean growth and yield attributes significantly reduced under drought stress at both pre and post flowering period, while maximum reduction was caused by PEG (16%) applied at pre flowering time. The endogenous bioactive GA1 and GA4 content decreased under elevated drought stress. On the other hand, jasmonic acid (JA), salicylic acid (SA) and abscisic acid (ABA) content increased under drought stress. On the basis of current study, we concluded that application of earlier drought stress severely reduced growth and yield attributes of soybean when compared to its later application. Furthermore, increases in the endogenous contents of JA, SA and ABA in response to drought stress demonstrate the involvement of these hormones in drought stress resistance.

Introduction

Drought is a worldwide problem and a major proportion of agriculture land is affected with varying degrees of drought. Water deficit, extreme temperatures and low atmospheric humidity lead to drought, which is one of the most limiting factors for better plant performance and higher crop yield (Szilgyi, 2003; Hirt & Shinozaki, 2003). The repercussions of water deficit include its adverse effects on plant phenology, phasic development, growth, carbon assimilation, assimilate partitioning and plant reproduction processes. Drought stress differentially affects the level of endogenous phytohormones. Phytohormones are naturally occurring organic substances, which influence physiological processes at low concentrations either in distant tissues to which they are transported or in the tissue where synthesis occurred (Davies, 1995a). Due to their structural simplicity, plant hormones are not specific enough to match the variety of controlled reactions (Canny, 1985). Contrary to this, it has been suggested that hormones only provide "turn on" or "turn off" signals and that the actual informations are provided by the cell. This scenario is similar to that of calcium, which is now thought to be an intermediate in some hormonal responses (Davies, 1995b).

*Corresponding author: ijlee@knu.ac.kr
The two hormones for which there is consistent evidence for endogenous regulation in response to environmental stress are ABA and ethylene (Gianfagna et al., 1992), although gibberellins, auxins and cytokinins are also implicated in stress response (Levitt, 1980; Narusaka et al., 2003). JA is reported to involve in diverse developmental processes, such as seed germination, root growth, fertility, fruit ripening and senescence (Creelman & Rao, 2002; Wasternack & Hause, 2002). JA activates plant defence mechanisms in response to insect-driven wounding, pathogens and environmental stresses including drought, low temperature and salinity (Wasternack & Parthier, 1997). SA application has resulted in tolerance of plants to many biotic and abiotic stresses including fungi, bacteria, viruses (Delany et al., 1994), chilling, drought and heat (Senaratna et al., 2003).

Soybean is the world foremost provider of protein and oil. It is often called the miracle crop as it contains high protein content (38–45%) as well as high oil content (20%). Water deficit adversely affects many physiological processes related to water use efficiency in soybean, thus leading to a decrease in plant productivity. Compared to other crops, soybean requires large quantities of water for a high yield (Heatherly, 1999; Sweeney et al., 2003). Nevertheless, most soybeans are cultivated under rain-fed conditions that are prone to drought. Water stress is detrimental to soybean growth throughout its development (Karam et al., 2005) and causes serious reduction in seed yield at the flowering and pod elongation stages because of flower and pod abortion (Liu et al., 2003). As the soybean plant ages from stage R1 (beginning bloom) through stage R5 (seed enlargement), its ability to compensate for stressful conditions decreases and the potential degree of yield reduction from stress increases (Foroud et al., 1993).

For drought stress induction, one of the most popular approaches is to use high molecular weight osmotic substances, like polyethylene glycol (PEG) (Turkan et al., 2005; Landjeva et al., 2008). In current study, PEG was used for drought stress induction in soybean. The physio-hormonal changes in response to drought stress were investigated, as our understanding of the hormonal response of soybean to drought stress is still limited.

Materials and Methods

General procedure: Seeds of famous soybean cultivar Hwangkeumkong were procured from Plant Genetics Lab., Department of Agronomy, Kyungpook National University. Hwangkeumkong is high yielding and widely cultivated soybean cultivar of Korea. Seeds were surface sterilized with 5% NaClO for 15 min., and then washed thoroughly with double distilled water. Initially 5 seeds were sown in pots (5.5 L) and were later thinned to 3 seedlings per pot on appearance of trifoliate. Horticulture soil was used as growth medium during this experiment. The composition of horticulture soil was as follows: peat moss (13-18%), perlite (7-11%), coco-peat (63-68%) and zeolite (6-8%), while the macro-nutrients were present as follows: NH$_4^+$~90 mg/L; NO$_3^-$~205 mg/L; P$_2$O$_5$~350 mg/L and K$_2$O~100 mg/L. The experiment comprised 4 replicates per treatment and each replication comprising of 9 plants.

Drought stress application: PEG (MW: 10,000) was used for induction of drought stress. The drought stress include three treatments i.e., control, moderate drought stress (8% PEG) and severe drought stress (16% PEG). Total of 3 doses of 300 ml of PEG solution was given to each pot with 3 days interval. The drought stress period comprised two weeks each, for both pre and post flowering treatments.
Growth and yield attributes of soybean: Growth parameters i.e., shoot length, shoot and root fresh and dry weights were measured for harvested soybean plants while chlorophyll content of fully expanded leaves was analyzed with the help of chlorophyll meter (Minolta Co., Ltd, Japan). Four replicates of 6 plants each per treatment were randomly selected for measuring growth and yield parameters. Dry weights were measured after drying the samples at 70°C for 48 h in an oven (Bohm, 1979).

Analysis of endogenous phytohormones: Plant samples were harvested at the completion of drought stress period and immediately frozen in liquid nitrogen and stored at minus 80°C. When all the required material for gibberellin (GA), abscisic acid (ABA), jasmonic acid (JA), and salicylic acid (SA) were prepared, the shoots were lyophilized in freeze drier (Virtis, SP Industries Inc.). The leaves were collected from lyophilized plant samples and crushed to powder for the analysis of plant hormones.

Analysis of bioactive gibberellins: The extraction was based on the already established procedure of Lee et al., (1998). Gas chromatograph-mass spectrometer (GC-MS) with selected ion monitoring (SIM) mode was used for the quantification of gibberellins. One μl of the extracted sample was injected in a 30 m × 0.25 mm (i.d.), 0.25 μm film thickness DB-1 capillary column (J & W Co., Folson, USA). The GC oven temperature was programmed for a 1 min., hold at 60°C, then to rise at 15°C min⁻¹ to 200°C followed by 5°C min⁻¹ to 285°C. Helium carrier gas was maintained at a head pressure of 30 kPa. The GC was directly interfaced to a Mass Selective Detector with an interface and source temperature of 280°C, an ionizing voltage of 70 eV and a dwell time of 100 ms. Retention time was determined by using the hydrocarbon standards to calculate the KRI (Kovats retention indices) value. Three replicates per treatment were used for determination of endogenous bioactive GA₁ and GA₄.

Analysis of endogenous free ABA: The endogenous ABA contents were extracted following the method of Qi et al., (1998) and Kamboj et al., (1999). The extracts were dried and methylated by adding diazomethane for GC-MS SIM (6890N network GC system and 5973 network mass selective detector; Agilent Technologies, Palo Alto, CA, USA) analysis. For quantification, the Lab-Base (ThermoQuset, Manchester, UK) data system software was used to monitor responses to ions of m/e 162 and 190 for Me-ABA and 166 and 194 for Me-[²H₆]-ABA.

Analysis of endogenous JA: The endogenous JA level was extracted according to the protocol of McCloud & Baldwin (1997). The extracts were analyzed with GC-MS SIM (6890N network GC system and 5973 network mass selective detector; Agilent Technologies, Palo Alto, CA, USA). To enhance the sensitivity of the method, spectra were recorded in the selected ion mode i.e. in case of JA determination, monitored the fragment ion at m/z= 83 amu corresponding to the base peaks of JA and [9, 10-²H₂]-9, 10-dihydro-JA (Koch et al., 1999). The amount of endogenous JA was calculated from the peak areas of endogenous JA in comparison with the corresponding standards. Three replicates per treatment were used for determination of JA.

Analysis of endogenous free SA: The free SA was extracted as described by Enyedi et al., (1992) and Seskar et al., (1998). SA was quantified with C18 reverse-phase HPLC (Waters Corp., Milford, MA, USA). The HPLC condition was maintained at fluorescence detector (Shimdzu RF-10AXL, with excitation 305 nm, and emission 365 nm).

Statistical analysis: The data was subjected to analysis of variance (ANOVA SAS release 9.1; SAS, NC, USA) and Duncan’s multiple range test (DMRT).
Table 1. Effect of PEG induced drought stress on growth of Soybean cv. Hwangkeumkong.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PEG level (%)</th>
<th>Shoot length (cm)</th>
<th>Fresh weight (g plant⁻¹)</th>
<th>Dry weight (g plant⁻¹)</th>
<th>Chlorophyll Content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Shoot Root Shoot Root Chlorophyll</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>105.4ᵃ</td>
<td>39.5ᵃ 15.8ᵃ 10.7³ a 4.85ᵃ 31.4ᵇ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-flowering stress (for 14 days)</td>
<td>8</td>
<td>86.6ᵇ</td>
<td>20.8ᵈ ed 7.33ᶜ 5.1ᶜ 2.45ᵇ 30.07ᵇ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-flowering stress (for 14 days)</td>
<td>16</td>
<td>81.1ᵇ</td>
<td>14.1ᵈ 6.12ᶜ 3.9ᶜ 2.13ᶜ 30.4ᵇ</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>85.9ᵃ</td>
<td>31.5ᵇ ab 13.9ᵇ ab 8.9ᵇ 4.72ᵃ 34.7ᵃ</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>72.6ᵇ</td>
<td>24.9ᵇ bc 11.7ᵇ ab 6.8ᵃ bc 4.1ᵇ 34.9ᵃ</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*In a column, treatment means having a common letter(s) are not significantly different at the 5% level by DMRT.

Table 2. Effect of PEG induced drought stress on yield of soybean cultivar Hwangkeumkong.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PEG level (%)</th>
<th>Pods/plant (number)</th>
<th>Pod D.W (g plant⁻¹)</th>
<th>100 seed wt. (g)</th>
<th>Yield (g plant⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>14.1ᵃ</td>
<td>5.4ᵃ</td>
<td>15.06ᵃ</td>
<td>5.18ᵃ</td>
</tr>
<tr>
<td>Pre-flowering (14 days)</td>
<td>8</td>
<td>8.5ᵇ ³</td>
<td>2.6ᵇ</td>
<td>13.9ᵃ</td>
<td>2.37ᵇ</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>6.3ᶜ</td>
<td>2.12ᵇ</td>
<td>10.3ᵃ</td>
<td>1.97ᵇ</td>
</tr>
<tr>
<td>Post-flowering (14 days)</td>
<td>8</td>
<td>11.5ᵇ ab</td>
<td>4.3ᵃ ab</td>
<td>14.4ᵃ</td>
<td>4.1ⁿ ab</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>10.6ᵃ bc</td>
<td>3.7ᵃ ab</td>
<td>14.3ᵃ</td>
<td>3.6ᵇ ab</td>
</tr>
</tbody>
</table>

*In a column, treatment means having a common letter(s) are not significantly different at the 5% level by DMRT.

Results and Discussion

Growth and yield components as influenced by drought stress: Drought stress is one of the important soybean growth limiting factor which decreases plant growth during vegetative stage. Many researchers believe that amount of crop water use determine plant growth and development. Meanwhile plants may injure under non optimal access of water at any stage (Daneshian & Zare, 2005; Daneshian & Jonobi, 2001). In current study, growth parameters markedly decreased due to PEG induced drought stress. Soybean growth was effected by PEG concentration and timing of stress application. Plant fresh weight and dry weight significantly reduced with 16% PEG, applied at pre-flowering growth stage. Plant shoot length insignificantly decreased in post flowering treatments than earlier doses of drought. The chlorophyll contents of leaves were least effected by drought stress, high chlorophyll contents per unit area was observed in drought stressed plants (Table 1). Current investigations confirm earlier reports that pointed plant height as a drought tolerance index and declared that tall soybean cultivar had more dry weight, and drought stress adversely affect all growth parameters (Daneshian & Jonobi, 2001).

The yield parameters also reduced drastically under elevated PEG induced drought stress. Mean numbers of pods/plant, pods dry weight, 100 seed weight and yield/plant were more severely affected by the pre-flowering application of drought stress than its later application. It was also observed that number of pods per plant was most sensitive to drought, while 100 seed weight was least affected by drought stress intensity and application time (Table 2).

Current results coincide with earlier reports, which stated that grain yield drastically reduced when drought occurs during flowering time (Hsiao, 1982; Boonjung & Fukai, 1996). Similarly, Datta et al., (1975) found that soil water stress during the vegetative stage reduced grain yields in by an average of 1500 kg/ha; whereas during the reproductive phase, yields were reduced by 2500 kg/ha, or more than 50%. Some researchers reported that soybean yield decreases with increase in drought stress. The yield reduction under stress condition may be due to the decline in number of grains per
pod caused by flower abscission during flowering stage (Daneshian & Zare, 2005; Daneshian & Jonobi, 2001).

**Influence of drought stress on bioactive gibberellins:** Gibberellins regulate all aspects of the life history of plants, from seed germination to vegetative growth and flowering (Ritchie & Gilroy, 1998). In current study, bioactive GA$_1$ and GA$_4$ contents were also significantly affected by drought stress. GA$_1$ concentration reduced with elevated PEG in both pre-flowering and post-flowering treatments. However, application of 8% PEG induced only a fractional decrease in GA$_1$ level of soybean. The GA$_4$ contents also decreased in a similar fashion in pre-flowering treatments, although in post-flowering plants, GA$_4$ levels were higher as compared to control. Higher quantity of endogenous GA$_4$ as compared to endogenous GA$_1$ contents showed that non C13-hydroxylation is the major GA biosynthesis pathway (Fig. 1). Present study confirms previous report on higher endogenous GA$_4$ production of soybean (Yoon et al., 2009).

**Influence of drought stress on JA:** Under drought stress conditions, the endogenous JA contents increased significantly with 8% PEG application (204.9ng/g), but reduced with double PEG (16%) dosage as compared to control (108ng/g), when exposed to pre-flowering osmotic stress regime. There is a marked reduction in JA concentration in response to basic and double PEG application during post-flowering period. The endogenous JA level in non stressed plants was higher at later stage of soybean growth and development (Fig. 2). Our current results coincide with previous reports that found a rapid increase in endogenous JA content in barley leaf segments subjected to osmotic stress induced with sorbitol or mannitol (Kramell et al., 2000; Lehmann et al., 1995).

**Influence of drought stress on SA:** Salicylic acid has enhanced tolerance of plants to many biotic and abiotic stresses including fungi, bacteria, viruses (Delaney et al., 1995), chilling, drought and heat (Senaratna et al., 2003). It appears that SA has a regulatory role in activating biochemical pathways associated with tolerance mechanisms (Sticher et al., 1997). Current study showed that under drought conditions, the endogenous SA content of soybean leaves significantly increased with the application of basic (8%) and double (16%) PEG during pre-flowering growth stage. However, increase in SA content was insignificant in plants that were exposed to PEG induced drought stress at post-flowering growth stage. We also observed that SA level was higher at reproductive stage than vegetative growth stage of soybean (Fig. 3).

**Influence of drought stress on ABA content:** This increase in ABA contents hinder soybean growth and development. It is due to the fact that ABA synthesized in plants upon drought stress, triggers closure of stomata thereby reducing water loss. Mechanically, the closure is facilitated by a reduction of the internal pressure (turgor) in the guard cells which is achieved by a concerted efflux of potassium ions and anions, sucrose removal and malate-conversion into osmotically inactive starch (Pei et al., 1998). We observed that soybean plant produce very high levels of ABA as compared to other endogenous plant hormones. The amount of ABA keep pace with growth of plant and maximum contents of ABA were found to produce at later stages of soybean growth and development. ABA contents of soybean leaves increased with the application of PEG induced drought stress. ABA level was also affected by PEG concentration, as ABA amount was much higher in double PEG (16%) applied soybean plants than with basic PEG application (Fig. 4).
Fig. 1. Endogenous GA₁ and GA₄ content of Hwangkeumkong leaves under drought stress.

Fig. 2. Endogenous JA content of Hwangkeumkong leaves under drought stress.
Fig. 3. Endogenous SA content of Hwangkeumkong leaves under drought stress.

Fig. 4. Endogenous ABA content of Hwangkeumkong leaves under drought stress.
Current results are in accordance with earlier reports, which narrated that ABA is involved in responses to environmental stress such as drought (Ober & Sharp, 2003). As the level of ABA increases during salt and drought induced reduction of water to plants, ABA has been thus postulated to play a central role in signalling for these stress responses (Zeevaart & Creelman, 1988).

Conclusion

Drought is a major constraint to agriculture production worldwide, as fertile lands are continuously rendered uncultivable due to drought stress. In current study, we showed that growth and yield attributes of soybean was adversely affected by PEG induced drought stress. Soybean plants were found to be more susceptible to an early drought stress as compared to drought stress at a later growth stage. The level of endogenous growth hormones was also affected by drought stress, as the contents of plant growth promoting hormone (gibberellin) declined, while those of JA and ABA increased under drought stress. It shows that JA and ABA are concerned with plant stress and reaffirms their role in plant resistance to abiotic stress. SA is related to systemic acquired resistance (SAR) of plant and an increase in the quantity of endogenous SA shows that soybean become more susceptible to injuries and pathogens under drought stress.

Acknowledgement

This work was financially supported by the Korea Research Foundation Grant (KRF-521-F00001) and Brain Korea 21, Republic of Korea.

References


(Received for publication 28 December 2009)