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Effect of exogenous methyl jasmonate on growth, gas exchange and chlorophyll contents of soybean subjected to drought

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Drought is considered as one of the major constraints to crop production worldwide. Methyl jasmonate (MJ) is a plant-signaling molecule that elicits a wide variety of plant responses ranging from morphological to molecular level. A pot-culture study was undertaken to investigate the possible role of MJ-treatment on growth, gas exchange and chlorophyll contents in soybean (Glycine max L. Merrill.) plants subjected to water stress. The soybean plants were grown under normal water supply conditions till blooming and were then subjected to moisture stress by withholding water followed by foliar application of MJ at the rate of 50 µM. Drought stress severely hampered the growth, leaf gas-exchange attributes as well as the photosynthetic pigment contents. It was evident from the experimental results that, MJ-treatment led to further impairment in growth by inhibiting the leaf gas exchange attributes and chlorophyll contents. It is worth noted that, MJ-treatment also hampered the performance of soybean crop under well-watered conditions. In all, MJ-treatment appeared to arrest the growth, impaired leaf gas-exchange attributes and caused the loss of chlorophyll contents of soybean plants under water deficit conditions.

Key words: Chlorophyll contents, drought stress, growth, gas exchange, soybean (Glycine max L. Merrill.).

INTRODUCTION

Environmental stresses trigger a wide variety of plant responses, ranging from altered cellular metabolism to changes in growth and plant productivity. Drought, salinity, chilling and extremes of temperature are among the major constraints to crop productivity. Among these, drought is a major abiotic factor that is becoming a serious threat to sustainable crop production worldwide. Drought refers to the absence of adequate moisture required for a plant to grow normally and complete its life cycle (Zhu, 2002).

Plants adaptation to abiotic stresses is very complex, due to the intricate of interactions between stress factors and various molecular, biochemical and physiological phenomena affecting plant growth and development (Razmjoo et al., 2008). The ability of crop plants to tolerate different abiotic stresses is directly or indirectly associated with their ability to acclimate at the level of photosynthesis, which in turn affects biochemical and physiological processes and consequently, the growth and yield of the whole plant (Chandra, 2003; Farooq et al., 2009). Changes in plant productivity due to alteration in gas exchange, especially photosynthetic rate, have received much attention worldwide. Many studies have
revealed the decreased photosynthetic activity under drought stress due to stomatal or non-stomatal mechanisms (Samarah et al., 2009). Stomatal closure deprives the leaves of CO₂ and photosynthetic carbon assimilation is decreased in favor of photorespiration. The "non-stomatal" mechanisms include changes in chlorophyll synthesis, functional and structural changes in chloroplasts and disturbances in processes of accumulation, transport and distribution of assimilates. Chloroplastic pigments are important to plants mainly for harvesting light. Loss of chlorophyll contents under water stress is considered a main cause of inactivation of photosynthesis (Blackburn, 2007). Photosynthetic pigments can be used as reliable indicators to evaluate the metabolic imbalance of photosynthesis and growth and yield performance under water deficit.

Soybean (Glycine max L. Merrill.) is considered a miracle crop due to its extraordinary qualities. It contains 40 to 42% good quality protein and 18 to 22% oil, so it is highly desirable in human diet (Arshad et al., 2006). Methyl jasmonate (MJ) is a naturally occurring plant growth regulator which can affect many morphological, physiological and biochemical processes in plants (Ueda and Saniewski, 2006; Norastehnia et al., 2007). MJ is generally considered to inhibit stomatal opening, cell division, plant growth and photosynthetic activities. Foliar application of MJ modulates several physiological responses, leading to improved resistance against abiotic stresses (Walia et al., 2007). However, the role of MJ in protecting plants from various abiotic stresses has been controversial. For instance, MJ has been observed to improve resistance against drought in rice (Lee et al., 2007); whereas its application caused substantial growth and yield reduction in rice (Kim et al., 2009). This dispute needs to be elucidated by monitoring the alteration in growth, gas exchange and chlorophyll contents. To the best of our knowledge, no study has been conducted in soybean in this regard. Therefore, this study was formulated to expound the role of exogenous application of MJ on growth, gas exchange and photosynthetic pigments in soybean under drought stress.

MATERIALS AND METHODS

The experiment was conducted during summer in 2009 in rain-protected wire-house at the College of Agronomy and Biotechnology, Southwest University, Chongqing, China. The experimental area lies between latitudes 29°49′32″N, longitudes 106°26′02″E, altitude 220 m and humid sub-tropical climate with average annual temperature of 18.7°C, relative humidity of 87.7% and 1090 mm rainfall.

Plant material and growth conditions

The seeds of soybean variety “Xidou-7” were planted in plastic pots (31 cm in diameter, and 24 cm in depth). This variety is extensively used in China in drought-related studies. A total of 7 seeds were sown per plastic pot and thinning was performed at first true leaf stage to maintain 3 plants per pot. The total weight of each pot was 12 kg filled with sandy loam soil containing organic matter of 25.76 g kg⁻¹, total nitrogen of 1.98 g kg⁻¹, total phosphorus of 1.77 g kg⁻¹, total potassium of 22.33 g kg⁻¹, available nitrogen of 55.71 mg kg⁻¹, available phosphorus of 27.55 mg kg⁻¹, available potassium of 88.47 mg kg⁻¹ and at pH 6.42. The pots were arranged in completely randomized design (CRB) with 20 pots per treatment and three replications of each experimental unit. All the pots were supplied with fertilizer at the rate of 10 g pot⁻¹ using NPK compound fertilizer with N: 15 %, P₂O₅: 5% and K₂O: 5% in two splits at planting and blooming stage. The temperature of the wire-house ranged from 19.2 to 37.6°C and relative humidity was 43.2 to 86.4% during the entire growth period.

Moisture stress and methyl jasmonate (MJ) application

Soybean plants were grown with normal water supply till blooming, and then were divided into four sets: (1) the set at 75% soil field capacity (well-watered) received distilled water; (2) the set at 75% soil field capacity (well-watered) received MJ application; (3) the set at 35% soil field capacity (drought) received distilled water and (4) the set at 35% soil field capacity (drought) received MJ application. Moisture treatments were regularly monitored by TRI-ME-EZ /-IT (IMKO Micromodultechnik GmbH, Germany). The pots were weighed daily to maintain the desired soil water contents by adding appropriate volume of water. MJ was foliar applied at the rate of 50 µM in 0.1% Tween-20 solution after one week of imposition of the moisture treatment at the blooming stage of soybean. A 20 ml aliquot was sprayed per plant.

Data measurement

The soybean plants were sampled (3rd leaf from top) after 5, 10 and 15 days of MJ application to determine the chlorophyll contents of the water stressed soybean plants. Leaf gas exchange attributes were measured after one week of MJ treatment. The growth related traits were assessed during the course of experiment and at harvesting.

Leaf gas-exchange

Net rate of photosynthesis (A), transpiration rate (E) and stomatal conductance (gₛ) were measured with a portable infrared gas exchange analyzer based photosynthesis system (LI-6400, Li-Cor, Lincoln, Nebraska, USA) during 8:00 to 10:00 am. 15 leaves were selected for each treatment with the following specifications: molar flow of air per unit leaf area was 389.62 mmol l⁻¹ m⁻² s⁻¹, water vapour pressure into leaf chamber was 3.13 mbar, PAR at leaf surface was up to 1199 mol m⁻² s⁻¹, leaf temperature ranged from 33.51 to 34.54°C, ambient temperature was 35.09 to 37.32°C, ambient CO₂ concentration was 358 mol mol⁻¹ and relative humidity (RH) was 59.32%.

Chlorophyll measurement

Fresh soybean leaf samples (0.10 g) were collected, cut into small pieces, ground and placed in 15 ml centrifuge tube, along with 10 ml of miscible liquids of 95.5% acetone and absolute ethyl alcohol in 1:1 ratio. Then the tube was covered with black plastic bag and kept in the dark for 48 h until the sample changed into white. Chl a, Chl b and Chl a+b concentrations were measured following the
Table 1. Influence of exogenous application of methyl jasmonate (MJ) on growth attributes of soybean under drought.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Plant height (cm)</th>
<th>Lowest node height (cm)</th>
<th>Leaf area (cm²)</th>
<th>Stem diameter (mm)</th>
<th>Number of node on main stem</th>
<th>Number of branch</th>
</tr>
</thead>
<tbody>
<tr>
<td>W</td>
<td>46.70±0.36</td>
<td>9.53±0.23</td>
<td>18.15±0.14</td>
<td>3.47±0.02</td>
<td>13.69±0.05</td>
<td>3.34±0.05</td>
</tr>
<tr>
<td>WMJ</td>
<td>45.61±0.32</td>
<td>9.09±1.11</td>
<td>17.06±0.20</td>
<td>3.58±0.03</td>
<td>13.29±0.07</td>
<td>3.51±0.03</td>
</tr>
<tr>
<td>D</td>
<td>44.88±0.19</td>
<td>8.34±0.27</td>
<td>15.09±0.23</td>
<td>2.98±0.05</td>
<td>11.59±0.06</td>
<td>2.49±0.04</td>
</tr>
<tr>
<td>DMJ</td>
<td>42.93±0.54</td>
<td>7.71±0.15</td>
<td>13.48±0.29</td>
<td>3.19±0.06</td>
<td>10.32±0.06</td>
<td>2.83±0.02</td>
</tr>
</tbody>
</table>

W, Well-watered; WMJ, methyl jasmonate (MJ) in well watered conditions; D, drought stress; DMJ, methyl jasmonate (MJ) in drought conditions. Values are mean ± SE (n = 3). Values followed by the same letter within columns were not significantly different according to Newman-Keuls test (P < 0.05).

method of Arnon (1949) by using UV-visible spectrophotometer and the absorbance was measured at 645, 652 and 663 nm respectively.

Growth related traits

Leaf area was measured with LI-3100 leaf area meter (Li-Cor, Lincoln, NE) CI-203 (CID, Inc., USA) after 15 days of MJ treatment. At harvest, 36 plants (12 plants from each replicate) representing each treatment were sampled randomly and quantified to determine the growth related traits.

Statistical analysis

The data set was statistically analyzed by the analysis of variance (ANOVA) technique using software SPSS 16.0. The main effects were analyzed and the means were compared by Newman-Keuls tests at a significance level of 0.05.

RESULTS

Growth related traits

The drought stress caused considerable inhibition of the growth attributes of water stressed soybean plants when compared with the well watered control. The observations demonstrated that, foliar application of MJ further inhibited the growth related traits in the drought-stressed soybean plants (Table 1). MJ-treatment in the water-deficit conditions decreased the plant height (4.54%), the lowest node height (8.17%), leaf area (11.94%), stem diameter (7.05%), number of nodes on main stem (12.31%), number of branches/plant (13.66%), height of lowest node (9.88%), nil-seed pod number (4.15%), one-seed pod number (11.70%), two-seed pod number (12.28%), three-seed pod number (11.28%) and four-seed pod number (39.65%) (Table 1). Exogenous MJ-treatment also hampered the growth related traits in the well watered control; however, the growth related inhibitory effect of MJ treatment was more pronounced in the water stressed plants than in the well watered control.

Leaf gas-exchange

The drought stress led to considerable decline in the net photosynthesis (16.68%), transpiration rate (23.49%), stomatal conductance (22.72%), water use efficiency (WUE) (17.68%), intercellular CO₂ (9.79%) and Ci/Ca (11.29%) when compared with the well watered control (Table 2). The foliar application of MJ led to further reduction in the gas exchange parameters of the water stressed as well as the well watered soybean plants. MJ-treatment reduced the net photosynthesis (11.03%), transpiration rate (23.36%), stomatal conductance (16.23%), WUE (11.86%), intercellular CO₂ (6.33%) and Ci/Ca (8.77%) in the water stressed soybean plants, but impaired the photosynthesis (4.28%), transpiration rate (7.96%), stomatal conductance (7.56%), WUE (6.39%), intercellular CO₂ (4.87%) and Ci/Ca (6.15%) under the well-watered plants (Table 2).

Photosynthetic pigments

The biosynthesis of photosynthetic pigments, including Chl a, Chl b and Chl a+b was noticeably reduced due to the water stress when compared with the well watered
Table 2. Influence of exogenous application of methyl jasomate (MJ) on leaf gas-exchange attributes of soybean under drought.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Net photosynthesis (A) (µmol m⁻² s⁻¹)</th>
<th>Transpiration rate (E) (mmol m⁻² s⁻¹)</th>
<th>Stomatal conductance (gs) (µmol m⁻² s⁻¹)</th>
<th>Water use efficiency (µmol mmol⁻¹)</th>
<th>Intercellular CO₂ (Ci) (µmol mol⁻¹)</th>
<th>Ci/Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>W</td>
<td>19.03±0.11 a</td>
<td>8.41±0.22 a</td>
<td>0.270±0.008 a</td>
<td>2.33±0.04 a</td>
<td>258±1.68 a</td>
<td>0.69±0.01 a</td>
</tr>
<tr>
<td>WMJ</td>
<td>18.25±0.25 a</td>
<td>7.79±0.17 b</td>
<td>0.251±0.006 a</td>
<td>2.19±0.02 ab</td>
<td>246±1.44 b</td>
<td>0.65±0.01 ab</td>
</tr>
<tr>
<td>D</td>
<td>16.31±0.35 b</td>
<td>6.81±0.11 c</td>
<td>0.222±0.007 b</td>
<td>1.98±0.06 bc</td>
<td>235±2.19 b</td>
<td>0.62±0.00 bc</td>
</tr>
<tr>
<td>DMJ</td>
<td>14.69±0.23 c</td>
<td>5.52±0.09 d</td>
<td>0.191±0.006 e</td>
<td>1.77±0.05 c</td>
<td>221±1.53 d</td>
<td>0.57±0.01 c</td>
</tr>
</tbody>
</table>

W: Well-watered; WMJ: methyl jasmonate (MJ) in well watered conditions; D: drought stress; DMJ: methyl jasmonate (MJ) in drought conditions. Values in the table are mean ± SE (n = 3). Values followed by the same letter within columns are not significantly different according to Newman-Keuls test (P < 0.05).

control as the water stress proceeded (Figure 1). MJ-treatment caused further reduction in Chl a contents by 5.31, 6.99 and 7.05%, Chl b contents up to 10.39, 8.22 and 6.97% and Chl a+b contents by 4.99, 5.49 and 4.34% under the drought conditions on the 5th, 10th and 15th day, respectively. Under normal (stress-free) conditions, the treatment of soybean plants with MJ also reduced the chlorophyll contents.

DISCUSSION

Drought, being the most important environmental stress, severely hampers plant growth and development, limits plant production and the performance of crop plants, more than any other environmental factor. It is now becoming evident that, methyl jasmonate can act as a true plant hormone, which mediates in various aspects of development and stress responses. It is biologically similar to abscisic acid and when applied to plants, evokes a wide variety of morphological, physiological and biochemical responses to stress (Creelman and Mullet, 1997). Growth is one of the most drought-sensitive processes due to the reduction in turgor pressure (Kusaka et al., 2005). In our study, moisture stress imposed at the blooming stage of soybean plants led to severe impairment in the growth related traits due to inhibition of cell expansion and cell growth owing to the low turgor pressure. Water deficits substantially reduced the plant height, the lowest node height, leaf area, stem diameter, number of nodes and branches plausibly by decreasing the soil’s water potential. The drought-induced reduction in the leaf area was also ascribed to suppression of leaf expansion through reduction in photosynthesis (Rucker, 1995). The reduction in plant height might be due to decline in the cell enlargement and more leaf senescence in the plant under water stress (Manivannan et al., 2007). The drought related reduction in the growth attributes of this study is in agreement with Hussain et al. (2008) which showed that water stress hampers mitosis, cell elongation and expansion which results in reduced growth traits. The exogenous application of MJ evoked major effects, indicating that MJ was responsible for the decline in the growth related traits. MJ-treatment led to considerable reduction in the growth of the drought-stressed soybean plants which is in agreement with Moore et al. (2003) which stated that, MJ arrested the growth and leaf expansion. Furthermore, these results are also consistent with Heijari et al. (2005) who reported that, seedling diameter, shoot fresh weight, root fresh weight and root length were hampered by MJ treatment in Scots pine. In this study, the drought stress severely hampered the leaf gas exchange parameters of soybean and this could be due to the decrease in leaf expansion, impaired photosynthetic machinery, premature leaf senescence, oxidation of chloroplast lipids and changes in the structure of pigments (Sgherri and Navari-Izzo, 1995). Stomata are highly sensitive to changes in soil water deficit, as conductance decreases quickly
Figure 1. Influence of methyl jasmonate (MJ) on Chl $a$, Chl $b$ and Chl $a+b$ contents in soybean under drought. W: Well-watered; WMJ: methyl jasmonate (MJ) in well watered conditions; D: drought stress; DMJ: methyl jasmonate (MJ) in drought conditions.
before much change in water potential (Atteya, 2003). MJ application might cause stomatal closure which inhibited CO₂ absorption and ultimately resulted in declined photosynthetic activity (Nayyar and Gupta, 2006). The MJ-induced reduction of the gas-exchange traits in conformity with Rossato et al. (2002), which revealed the inhibitory role of MJ on gas-exchange. This also agrees with the studies of Hristova and Popova (2002), who observed a decline in the photosynthesis of barley leaves, but it is in contrast to the findings of Suhita et al. (2003). The transpiration rate of soybean plants submitted to MJ treatment exhibited slight reduction as reported previously in barley (Horton, 1991). These results suggested that, MJ is the key component for the observed inhibition in drought-stressed soybean plants.

The decrease in chlorophyll content under drought stress has been considered a typical symptom of oxidative stress and may be the consequence of pigment photo-oxidation and chlorophyll degradation. Chlorophyll loss has been considered the principal criterion of senescence in most reports. The drought-induced reduction of the chlorophyll contents are in agreement with those of Anjum et al. (2011). The drought-induced reduction in the chlorophyll content could be attributed to loss of chloroplast membranes, excessive swelling, distortion of the lamellae vesiculation and the appearance of lipid droplets. The decrease in chlorophyll contents by MJ-treatment in our study is in accordance with that of Jung (2004), who reported the significant loss of pigment contents due to MJ-treatment. This MJ-induced loss of chlorophyll contents could be attributed to the MJ-promoted leaf senescence (He et al., 2002). MJ-induced loss of photosynthetic pigments would decrease the amount of energy absorbed by the photosynthetic apparatus, thereby attenuating energy requiring abiotic events such as photosynthesis.

**Conclusions**

Drought is a worldwide problem, constraining global crop production seriously and recent global climate change has made this situation more serious. It is evident from our results that, drought led to substantial reduction in growth, gas-exchange and chlorophyll contents, whereas MJ-treatment caused further inhibition of growth, chlorophyll contents and photosynthetic activity.

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