Counteraction of Drought Stress on Soybean Plants by Seed Soaking in Salicylic Acid

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Abstract: The interactive effects of drought stress (70, 50 and 30% field capacity) and salicylic acid (0.6 mM) were studied in soybean (Glycine max L.) plants. The content of pectin, cellulose, lignin and phospholipids of either shoots or roots and soluble sugars of shoots were significantly lowered with the rise of drought levels. On the other hand, the contents of hemicellulose, starch, total lipids, glycolipids and sterols of either shoots or roots, soluble sugars of roots were raised. It was found that application of salicylic acid enhanced pectin, cellulose, lignin and phospholipids contents of either shoots or roots and soluble sugars of roots. On the other side, the applied salicylic acid lowered hemicellulose, starch, total lipids, glycolipids and sterols of shoots and roots and soluble sugars of shoots. Soaking of soybean seeds in salicylic acid exhibited a favorable effect on the accumulation of some ions and antagonized or ameliorated the inhibitory effect of drought stress on some others.

Key words: Pectin, hemicellulose, cellulose, lignin, glycolipids, phospholipids, sterols, minerals, Glycine max

INTRODUCTION

The physiological and biochemical responses of plants to drought stress may be achieved by alteration of certain phases of the metabolic network mediated and directed by certain enzymes, which activate or retard particular metabolic activities. This may divert the perfect balance of the biochemical reactions with the accumulation of some metabolic intermediates or end products.

Salicylic acid, an ubiquitous plant phenolic, was recognized as an endogenous regulator in plants after the finding that it is involved in many plant physiological processes. In an extensive screening program using modern analytical technique, salicylic acid was detected in the leaves and reproductive organs of 34 agronomically important species (Raskin et al., 1990).

One of the most studied functions of salicylic acid is that it is associated with its involvement in plant resistance response to different pathogen attacks (Yalpani and Raskin, 1993; Alvarez, 2000). It was also reported that salicylic acid accumulates during exposure to ozone or UV light (Sharma et al., 1996). In this respect salicylic acid treatment was found to improve the chilling, heat-shock, drought and salt (Janda et al., 1999; Guo-Zhang et al., 2004; Dat et al., 1998; 2000; Hamada and Al-Hakimi, 2001; Szalai et al., 2005). Thus, in this study it seemed necessary to study the effects of various levels of drought on some metabolic processes (cell wall polysaccharides, soluble sugars, starch, total lipids, glycolipids, phospholipids, sterols and mineral composition) and the role of salicylic acid in amelioration of the adverse effects of drought.

MATERIALS AND METHODS

The present study was conducted in greenhouse at Taiz University, Biology Department at summer 2005. The mean temperature was about 27°C and 70% relative humidity. Seeds of soybean (Glycine max L.) were grown in plastic pots (10 cm in diameter and 13.5 cm in height) lined with polyethylene bags and containing soil composed of clay and sand (2:1). The seeds of soybean before sowing were soaked for 6 h in solutions containing 0.6 mM salicylic acid and the other seeds soaked for 6 h in distilled water (regarded as control). After sowing seeds (5 seed in each pot), the pots then irrigated and the water content of the soil was adjusted regularly near to the field capacity and the plants were left to grow for 15 days. Thereafter, the pots (four replicate per treatment) were watered to the desired soil moisture content (70, 50 and 30% field capacity). Some pots were left untreated (100% field capacity and 0 salicylic acid) and regarded as absolute control. On the other side, droughted plants but non-treated with salicylic acid were regarded as reference control. At the end of the experimental period (30 days) cell wall fractionation was conducted essentially according to Dever et al. (1968) and Galbraith and Shields (1981). Tissue powder samples were extracted twice in distilled water, twice in 80% ethanol to
remove soluble sugars metabolites. The residue was then extracted in 2 mL 0.5 N NaOH for starch, 0.5% ammonium oxalate-oxalic acid (50°C for 24 h) for pectin, 17.5% NaOH for hemicellulose and in 72% H2SO4, (with 15 min autoclav) for cellulose extraction. After that, the remaining precipitate was aserbed to the lignin fraction according to Dever et al. (1968). Contents of soluble sugars, starch and wall polysaccharides were determined by the anthrone sulfuric acid reagent using glucose as standard (Fales, 1951). Lipids were extracted three times for dried plant organs (each lasted for 24 h) with chloroform/methanol (2:1, v/v) at room temperature according to Navari-Izzo et al. (1989). Lipids (100 μg) were chromatographed on silica gel plates (silica gel G/60) using n-hexine-diethylether-glacial acetic acid (70: 30: 1) and the spots were visualized by I2 vapor. According to Navari-Izzo et al. (1989) the Total Polar Lipids (TPL) were located at the origin of the chromatogram. These were scratched and redissolved in chloroform/methanol (2:1). Glycolipids were estimated as their monosaccharides content by the anthrone sulfuric acid reagent. Phospholipids were determined in lipid extracts according to Johnson (1971). Lipid phosphorus was estimated by the molybdate blue colour (Woods and Mellon, 1941). Phosphorus content was taken as an index for phospholipid contents. Total sterols were estimated according to the method described by Cook (1958). Sodium and potassium were determined by flame photometer method (Williams and Twine, 1960), calcium and magnesium by the versene titration method (Schwarzenbach et al., 1948).

All data presented are the mean values. Statistical analysis was carried out by one-way ANOVA using Student’s t-test to test significance of the difference between means. Means were considered significantly different at 5 or 1% level of probability.

**RESULTS AND DISCUSSION**

Cell wall metabolism is an important component in plant growth, not only because it represents a large proportions of the cell biomass, but also because determining wall extensibility for cell enlargement (Zhong and Läuchli, 1988). In this investigation, pectin, cellulose, hemicellulose and lignin were determined in stressed soybean plants (Table 1 and 2). The contents of pectin, cellulose and lignin of shoots and of roots were significantly lowered by drought level. On the other side, hemicellulose contents of either shoots or roots were raised under all the drought levels. This could be important for preventing extension (Taiz, 1984; Volkenburgh and Boyer, 1985). Working with tobacco cell cultures in the presence of polyethylene glycol, Iraki et al. (1989) recorded a decrease in the percentage of cellulose and an increase in the hemicellulose percentage, whereas the pectic fraction remained more or less unchanged. Similar results were, also obtained by Wakabayashi et al. (1997). The possible mechanisms for the inhibitory effect of salinity on the incorporation of glucose into cell wall polysaccharides have been discussed (Zhong and Läuchli, 1988; Hamada, 2001; Al-Hakimi, 2005).
Table 4: The effect of Salicylic Acid (SA) treatment in ameliorating the adverse effects of drought stress on the content of total lipids, glycolipids, phospholipids and sterols (mg g⁻¹ (d.m.)) of shoots of soybean plants.

<table>
<thead>
<tr>
<th>Drought-SA (as PC - mM)</th>
<th>Total lipids</th>
<th>Glycolipids</th>
<th>Phospholipids</th>
<th>Sterols</th>
</tr>
</thead>
<tbody>
<tr>
<td>100+0</td>
<td>31.229</td>
<td>10.976</td>
<td>1.117</td>
<td>3.153</td>
</tr>
<tr>
<td>70+0</td>
<td>39.227</td>
<td>16.377</td>
<td>0.572</td>
<td>5.377</td>
</tr>
<tr>
<td>50+0</td>
<td>42.136</td>
<td>17.902</td>
<td>0.444</td>
<td>7.877</td>
</tr>
<tr>
<td>30+0</td>
<td>49.170</td>
<td>22.973</td>
<td>0.316</td>
<td>8.497</td>
</tr>
<tr>
<td>100+0.6</td>
<td>21.083</td>
<td>8.042</td>
<td>1.612</td>
<td>2.123</td>
</tr>
<tr>
<td>70+0.6</td>
<td>18.972</td>
<td>6.367</td>
<td>1.472</td>
<td>2.837</td>
</tr>
<tr>
<td>50+0.6</td>
<td>20.809</td>
<td>7.267</td>
<td>1.803</td>
<td>4.283</td>
</tr>
<tr>
<td>30+0.6</td>
<td>23.861</td>
<td>8.592</td>
<td>1.972</td>
<td>5.379</td>
</tr>
<tr>
<td>LSD at 5%</td>
<td>1.853</td>
<td>1.180</td>
<td>0.097</td>
<td>0.155</td>
</tr>
<tr>
<td>LSD at 1%</td>
<td>2.696</td>
<td>1.717</td>
<td>0.141</td>
<td>0.226</td>
</tr>
</tbody>
</table>

*Significant (p = 0.05), **highly significant (p = 0.01) as compared with control

Table 5: The effect of Salicylic Acid (SA) treatment in ameliorating the adverse effects of drought stress on the content of total lipids, glycolipids, phospholipids and sterols (mg g⁻¹ (d.m.)) of roots of soybean plants.

<table>
<thead>
<tr>
<th>Drought-SA (as PC - mM)</th>
<th>Total lipids</th>
<th>Glycolipids</th>
<th>Phospholipids</th>
<th>Sterols</th>
</tr>
</thead>
<tbody>
<tr>
<td>100+0</td>
<td>21.313</td>
<td>5.593</td>
<td>0.654</td>
<td>2.850</td>
</tr>
<tr>
<td>70+0</td>
<td>29.023</td>
<td>7.632</td>
<td>0.316</td>
<td>4.702</td>
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<tr>
<td>50+0</td>
<td>30.894</td>
<td>8.681</td>
<td>0.285</td>
<td>5.752</td>
</tr>
<tr>
<td>30+0</td>
<td>36.225</td>
<td>9.797</td>
<td>0.213</td>
<td>7.389</td>
</tr>
<tr>
<td>100+0.6</td>
<td>16.197</td>
<td>3.773</td>
<td>0.888</td>
<td>1.957</td>
</tr>
<tr>
<td>70+0.6</td>
<td>14.107</td>
<td>3.118</td>
<td>0.787</td>
<td>1.705</td>
</tr>
<tr>
<td>50+0.6</td>
<td>15.392</td>
<td>2.587</td>
<td>0.856</td>
<td>3.394</td>
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<td>30+0.6</td>
<td>17.037</td>
<td>2.122</td>
<td>0.936</td>
<td>4.617</td>
</tr>
<tr>
<td>LSD at 5%</td>
<td>1.543</td>
<td>0.385</td>
<td>0.017</td>
<td>0.163</td>
</tr>
<tr>
<td>LSD at 1%</td>
<td>2.246</td>
<td>0.560</td>
<td>0.025</td>
<td>0.238</td>
</tr>
</tbody>
</table>

*Significant (p = 0.05), **highly significant (p = 0.01) as compared with control

The adverse effects of soil moisture content on pectin, cellulose and lignin contents in shoots and roots were partially or completely alleviated by soaking seeds in salicylic acid. The applied salicylic acid inhibited the stimulatory role of water stress on the production of hemicellulose in shoots and roots. Salicylic acid could perhaps alleviate the inhibitory effects of drought on glucose incorporation into cell wall polysaccharides. Similarly cellulose biosynthesis was particularly enhanced (Zhong and Lauhli, 1988). These authors suggested that high Na⁺ concentrations reduced cellulose biosynthesis in cotton roots via disturbance of plasma membrane integrity. In this study treatment with salicylic acid, however, counteract this adverse effect (Table 1 and 2).

Drought stress stimulated soluble sugars accumulation in roots and retarded their biosynthesis in shoots (Table 3). The applied salicylic acid enhanced the stimulatory role of water stress on accumulation of soluble sugars in roots in drought stressed soybean plants. Also in this context, the applied salicylic acid retarded the accumulation of soluble sugars in shoots (Table 3). The starch was accumulated at all investigated drought levels in shoots and roots. The applied salicylic acid was generally effective in antagonizing parially or completely the stimulatory effect of drought stress on starch accumulation in shoots and roots of test plants. Gordon et al. (1986) showed that regrowth of defoliated white clover was associated with a decrease in starch and other sugars of shoots and roots. It is accepted that with the demand for sugars starch degradation increased, but that was not strictly associated with low concentrations of sucrose, glucose and fructose ( Bauer-Hoch et al., 1990).

Water stress was found to affect the incorporation of internally available sugars into various fractions of cell wall in excised root segments or cell suspension cultures ( Iraki and Carpta, 1986; Solomon et al., 1987; Al-Hakimi and Hamada, 2001; Al-Hakimi, 2005).

Lipid and lipid fractions of the studied plants exhibited variable changes in response to the drought stress and their interaction with the applied salicylic acid (Table 4 and 5). Total lipid and glycolipid contents of shoots and roots were significantly increased with the decrease in soil moisture content. The phospholipid fraction was significantly lowered by drought stress. Total sterols were accumulated in drought-stressed shoots and roots. Salicylic acid treatment retarded the total lipid, glycolipid and total sterols of shoots and roots.

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On the contrary, salicylic acid stimulated the phospholipids accumulation in shoots and roots of droughted plants. Similar decrease or increase in lipid contents by stress imposition was also recorded in plants (Peeler et al., 1989; Dakhma et al., 1995; Quartacci et al., 1997; Al-Hakimi, 2003, 2005).

The decrease in phospholipids may be due either to reduction in the power of hydrophobic proteins to bind phospholipids (Heber and Sanatarius, 1964) or to lipid peroxidation on enhanced lecithinase (Harrison and Trevelyan, 1963). In this respect, Martin et al. (1986) and Al-Hakimi (2003) reported a general decrease in phospholipid and glycolipid contents with decreasing soil moisture content. The phospholipid loss is also known to occur under several adverse climatic conditions such as dehydration (Graziane and Livne, 1971; Quartacci et al., 1997), chilling (Lurie et al., 1994), freezing (Lea and Hawke, 1952), drought (Navari-Izzo et al., 1993; Al-Hakimi, 2003) and senescence (Simon, 1974; Brown et al., 1994). Sterols also play essential functions in the lipid core of cell membrane and they are also biogenetic precursors of numerous secondary metabolites including plant steroid hormones (Geuns, 1978). It has been shown that sterols could influence the structural and functional properties of biological membranes (Horvath et al., 1981).

Significant response in connection with the interaction of drought and salicylic acid treatments was manifested in the present investigation with respect to ionic balance which is considered as one of the most complicated and integral parts of plant activities. The cation imbalance is one of the most basic disorders due to drought stress. The results of the present study (Table 6 and 7) reveal unequivocally that the applied drought stress was effectual in producing Na⁺ accumulation in the different organs of soybean plants; the highest Na⁺ accumulation was consistently displayed in plants subjected to the highest drought level. Serrano and Oxiola (1994) reported that the high concentration of Na⁺ negatively affected the intercellular K⁺ accumulation, presumably either by competing for sites through which influx of both cations occurs (Jeschke and Wolf, 1988) or affecting membrane stability causal leakage of K⁺ (Watan et al., 1991). The alterations in distribution and accumulation of mono-and divalent cations in the different organs of drought stressed plants may be an indication of the role of these cations in regulating the physiological activities of these plants (Benzioni et al., 1992). The accumulation of K⁺ and Mg²⁺ decreased gradually with the rise of drought level stress and this trend was generally accompanied by reciprocal variations in the concentration of calcium. Variations in the concentration of K⁺ and Mg²⁺ in soybean plants due to increased drought levels were generally accompanied by reciprocal variation in the concentration other mono (Na⁺) and divalent (Ca²⁺) cations, i.e., Na⁺/K⁺ and Ca²⁺/Mg²⁺ ratios increase along with drought level; a trend which is in accordance with some of the results of Garcia-Reina et al. (1988) and Torres-Schumann et al. (1989) using other plant tissues.

Seed soaking in salicylic acid had an inhibitory effect on the accumulation of sodium in the different organs under various levels of drought. Furthermore, their application ameliorated the inhibitory effects of drought on K⁺ and Mg²⁺ accumulation in the different organs of the test plants. Also, this treatment enhanced the stimulatory effect of drought stress on the accumulation of Ca²⁺ in soybean organs. The salicylic acid and ubiquitous plant phenolic compound, was recognized as an endogenous regulator in many plant physiological processes (Enyedi et al., 1992; Yalpani and Raskin, 1993). In this context, Janda et al. (1999) observed that salicylic acid pretreatment at normal growth temperature induced protection against low-temperature stress in young maize plants, probably due to increased antioxidant activity. Also, Mishra and Choudhuri (1999) found that deterioration at heavy metal stress was partially alleviated by the exogenous application of salicylic acid in Oryza sativa.

REFERENCES


