Sodium nitroprusside and salicylic acid decrease antioxidant enzymes activity in soybean

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Abstract

The present study investigated the effect of salicylic acid (SA) and sodium nitroprusside (SNP) on germination and activity of antioxidant enzymes in soybean under oxidative stress using a factorial experiment based on completely randomized design with three replications. Treatments included SA (0 and 1 mM), SNP (0, 30, and 60 µM), and H₂O₂ (0, 50, and 100 µM). Results showed that the highest germination percentage (97%) was related to 1 mM SA without SNP and H₂O₂ which was 6% greater than the control and the highest germination rate (0.72) was related to 1 mM SA and 60 µM SNP without H₂O₂ (6.63% greater than the control). The highest activity for superoxide dismutase (89.17 units mg⁻¹ protein) was achieved by application of 100 µM H₂O₂ without SNP. In conclusion, oxidative stress increased all antioxidant enzymes while SNP application decreased the enzyme activity and stress severity.

Keywords: soybean; germination; hydrogen peroxide; oxidative stress; catalase


Introduction

Oxidative injuries pose limitation for growth and production in plants. Reactive oxygen species (ROS) may have harmful effects like lipid peroxidation, changes in cell membrane integrity, and consequently disintegration of its structure (Allen, 1995).

Salicylic acid (SA) is a plant hormone which regulates the antioxidant mechanisms of plants and defense against oxidative stress (Singh Gill and Tuteja, 2010). SA has inhibitory effects on catalase activity and subsequently on establishing the defense mechanisms in plants (Durant and Dong, 2004).

Sodium nitroprusside (SNP) is a nitric oxide (NO) releasing compound (Zheng et al., 2009) that participates in some physiological processes in plants like germination, ROS metabolism, and signal transduction (Nill et al., 2002). Under biotic and abiotic stresses, the concentration of various ROS, e.g. superoxide, hydroxyl radical, and H₂O₂ increases in plants (Streeter et al., 2001). ROS damage cell membrane and chloroplasts and macromolecules such as proteins, nucleic acids, and lipids (Mittler, 2002). Therefore, this experiment was conducted to study the effect of SA and SNP on germination and its related traits in soybeans under oxidative stress.
Comparison of physiological traits of soybeans under the influence of SA, SNP, and oxidative stress caused by H$_2$O$_2$

<table>
<thead>
<tr>
<th>SA (mM)</th>
<th>SNP (µM)</th>
<th>H$_2$O$_2$ (µM)</th>
<th>GP (%)</th>
<th>GR</th>
<th>GU</th>
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<tr>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>3.25 ± 0.1</td>
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<td>0.27 ± 0.011</td>
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<tr>
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<td>0.58 ± 0.01</td>
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</table>

SA (salicylic acid), SNP (sodium nitroprusside), H$_2$O$_2$ (hydrogen peroxide), GP (germination percentage), GR (germination rate), GU (germination uniformity)

**Materials and Methods**

A factorial experiment was conducted to investigate the effect of SA and SNP on soybean (cv. Katul) seed germination under oxidative stress based on completely randomized design with three replications in 2017. Treatments consisted of SA (0, 1 mM), SNP (0, 30, and 60 µM), and H$_2$O$_2$ (0, 50, and 100 µM). Seeds were surface sterilized with sodium hypochlorite 10% and rinsed with distilled water. Then, 25 seeds were planted between paper in Petri dishes and treatment solutions were added. Petri dishes were placed in a germinator (25 ± 1 °C) for 8 days and germinated seeds were counted daily.

Germination percentage (GP), germination rate (GR), mean germination time (MGT), and germination heterogeneity (GU) were calculated using GERMIN program. Seedling growth test was conducted and on day 5 of the study, five seedlings were selected from each treatment and placed in liquid nitrogen immediately for measuring the activity of antioxidant enzymes.

Catalase (EC: 1.11.1.6) activity assayed according to Chance and Maehly procedure (1955). Also, peroxidase (EC: 1.11.1.7) activity was measured according to the method of MacAdam et al. (1992). Furthermore, superoxide dismutase (EC: 1.15.1.1) activity was assayed according to Sen Gupta et al. (1993) method. Data were subjected to analysis of variance using SAS 9.1 software. Means were compared by least significant differences (LSD) test at 5% probability level.

**Results**

The highest GP (97.67%) was observed by using 1mM SA without SNP and H$_2$O$_2$ treatment and GP (84/33%) was observed by 30 µM SNP and 50 µM H$_2$O$_2$ (Table 1). The highest GR (0.72) was obtained by 1mM SA and 60 m SNP without application of H$_2$O$_2$ which was significantly different from 1 mM SA, 60 µM SNP and 50 µM H$_2$O$_2$ that was 63.6% greater than control and GR (0.63%) was obtained by 1mM SA, 30 µM SNP and 50 µM H$_2$O$_2$ (Table 1). The lowest MGT (1.48 day) was achieved by application of 1 mM SA without H$_2$O$_2$ (Table 2) which was 41.9% reduction compared with control and MGT (1.8 day) observed in the treatment with 60 µM SNP and 50 µM H$_2$O$_2$. The lightest GU (1.66) was observed in the application of 60 µM SNP while GU (2.85) was
obtained by 1 mM SA, 30 µM SNP, and 50 µM H$_2$O$_2$ (Table 1). The highest SOD (89.17 units mg$^{-1}$ protein) and POX activities (102.5 units mg$^{-1}$ protein) were observed by using 100 µM H$_2$O$_2$ without SNP and SOD (48 units mg$^{-1}$ protein) and POX activities (82.17 units mg$^{-1}$ protein) was observed in the treatment involving 50 µM H$_2$O$_2$ and 30 µM SNP (Table 3). The highest activities of SOD, CAT, and POX (70.78, 18.78, and 97.22-unit mg$^{-1}$ protein, respectively) were observed in the treatment with 1 mM SA without SNP (Table 4). Analysis of variance of the effect of treatments on soybean seed germination traits and antioxidant enzymes were shown in Tables 5 and 6.

### Discussion

H$_2$O$_2$ is the free radical especially in cell membranes which causes oxidative stress while SA decreases the severity of oxidative stress and increases the proline content (Shi et al., 2009). Protecting plants against ROS was related to SA and its contribution in the synthesis of antioxidant enzymes and activity of the enzymes (Wu and Du, 2008). SA can control the activity of antioxidant enzymes through the temporary accumulation of ABA (Hayat and Ahmad, 2007). SA reduces the activity of catalase at the early stages of stress and increases concentration of H$_2$O$_2$ which acts as a secondary messenger for the activation of resistance genes (Hernandez et al. 2001).

SNP is an NO donor which participates in transduction pathway and contributes in some processes like germination, root growth, and stomatal closure (Zheng et al. 2009). Positive effects of SNP on seed germination was reported by Fan et al. (2012) in Cacumis satirus. NO releasing compounds have the capability to fight ROS and reduce oxidative damages (Asadi Karam et al., 2016). Effect of NO is dose-dependent and its physiological effects varies at different concentrations and in environmental conditions (Shi et al. 2009).

GR is one of the most important indices among agronomic characters and low GR leads to a lack of homogeneity and density under field conditions. Homogeneity and germination rate of wheat was reported to decrease under drought stress (Fateh et al. 2012). In conclusion, under
oxidative stress 60 µM of SNP is recommended to use in soybeans in order to have a better germination and seedling emergence.

References


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