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 H_2O_2 (0, 50, and 100 µM). Results showed that the highest germination percentage (97%) was related to 1 mM SA without SNP and H_2O_2 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 H_2O_2 (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 H_2O_2 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

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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 radicle, and H_2O_2 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.

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Table 1
Comparison of physiological traits of soybeans under the influence of SA, SNP, and oxidative stress caused by H_2O_2

SA (mM)	SNP (μM)	H_2O_2 (μM)	GP (%)	GR	GU
0	0	0	91.67 ± 1.53	0.44 ± 0.01	3.25 ± 0.1
		50	85 ± 1	0.33 ± 0.21	4.1 ± 0.07
		100	71 ± 1	0.27 ± 0.011	5 ± 0.1
	30	0	89.33 ± 1.53	0.45 ± 0.01	2.46 ± 0.07
		50	84.33 ± 2.1	0.36 ± 0.02	3.53 ± 0.11
		100	79.67 ± 1.53	0.31 ± 0.01	4.18 ± 0.03
	60	0	91.33 ± 1.53	0.57 ± 0.02	1.66 ± 0.05
		50	86.33 ± 1.53	0.47 ± 0.02	2.29 ± 0.07
		100	74.67 ± 1.53	0.41 ± 0.01	3.45 ± 0.08
1	0	0	97.67 ± 1.53	0.64 ± 0.01	2.22 ± 0.08
		50	93.67 ± 1.53	0.52 ± 0.005	2.53 ± 0.13
		100	90.33 ± 1.53	0.45 ± 0.004	2.6 ± 0.06
	30	0	96.67 ± 1.53	0.67 ± 0.01	3.14 ± 0.1
		50	93 ± 2	0.63 ± 0.10	2.85 ± 0.13
		100	90 ±1	0.53 ± 0.01	2.23 ± 0.08
	60	0	95.33 ± 1.53	0.72 ± 0.02	1.84 ± 0.11
		50	92.33 ± 0.58	0.68 ± 0.01	2.2 ± 0.06
		100	88.33 ± 1.53	0.58 ± 0.01	1.71 ± 0.08

SA (salicylic acid), SNP (sodium nitroprusside), H_2O_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_2O_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_2O_2 treatment and GP (84/33%) was observed by 30 μM SNP and 50 μM H_2O_2 (Table 1). The highest GR (0.72) was obtained by 1mM SA and 60 m SNP without application of H_2O_2 which was significantly different from 1 mM SA, 60 μM SNP and 50 μM H_2O_2 that was 63.6% greater than control and GR (0.63%) was obtained by 1mM SA, 30 μM SNP and 50 μM H_2O_2 (Table 1). The lowest MGT (1.48 day) was achieved by application of 1 mM SA without H_2O_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_2O_2. The lightest GU (1.66) was observed in the application of 60 μM SNP while GU (2.85) was

Table 2
Comparison of means for the interaction of SA and H_2O_2 on MGT (mean of germination time) in soybean

SA (mM)	H_2O_2 (µM)	MGT (day)
0	0	0.25 ± 2.1
	50	0.43 ± 2.65
	100	0.54 ± 3.11
1	0	0.07 ± 1.48
	50	0.20 ± 1.65
	100	0.23 ± 1.93

SA (Salicylic acid), H_2O_2 (hydrogen peroxide)

Table 3
Comparison of means for the interaction of SNP and H_2O_2 on soybean antioxidant enzymes

SNP (µM)	H_2O_2 (µM)	SOD (unit mg^{-1} protein)	POX (unit mg^{-1} protein)
0	0	6±38.5	7.42±79.33
	50	6.99±67	8.31±88.5
	100	7.86±89.17	8.48±102.5
30	0	6.19±34.5	6.54±74
	50	3.22±48	9.04±82.17
	100	4.62±58.17	10.85±91.17
60	0	5.19±28.83	9.18±61
	50	7.76±35.83	10.94±72
	100	10.13±44.67	11.17±82.5

SOD (superoxide dismutase), POX (peroxidase)

Table 4
Comparison of means for the interaction of SA and SNP on soybean antioxidant enzymes

SA (mM)	SNP (µM)	SOD (unit mg^{-1} protein)	CAT (unit mg^{-1} protein)	POX (unit mg^{-1} protein)
0	0	21.3±59	4.37±13.89	9.84±83
	30	11.36±43.11	4.07±9.89	5.83±74.55
	60	5.17±29.67	4.74±10	8.63±62.78
1	0	22.97±70.78	5.12±18.78	10.78±97.22
	30	9.7±50.67	3.24±17.33	9.38±90.33
	60	9.11±43.22	2.71±14.89	10.24±81.22

SOD (superoxide dismutase), POX (peroxidase), CAT (catalase)

obtained by 1 mM SA, 30 µM SNP, and 50 µM H_2O_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_2O_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_2O_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_2O_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_2O_2 which acts as a secondary messenger for the activation of resistance genes (Hernandez et al. 2001).

SNP is an NO donator 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

Table 5
Analysis of variance of the effect of treatments on soybean seed germination traits

Sov	Df	GP	GR	GU	MGT
SA	1	1176^{**}	0.55^{**}	12.33^{**}	11.55^{**}
SNP	2	3.02^{ns}	0.77^{**}	5.98^{**}	1.82^{**}
H_2O_2	2	585.41^{**}	0.11^{**}	2.73^{**}	2.47^{**}
SA × SNP	2	14.39^{**}	0.005^{**}	1.73^{**}	0.4^{**}
SA × H_2O_2	2	92.67^{**}	0.002^{**}	4.46^{**}	0.39^{**}
SNP × H_2O_2	4	15.85^{**}	0.001^{**}	0.18^{**}	0.11^{**}
SA × SNP × H_2O_2	4	12.22^{**}	0.0004^{*}	0.22^{**}	0.01^{ns}
Error	36	2.2	0.0002	0.008	0.006
CV%		1.68	2.6	3.15	3.75

ns, *, and ** are non-significant, and significant at 0.05 and 0.01% probability levels, respectively. SA (salicylic acid), SNP (sodium nitroprusside), H_2O_2 (hydrogen peroxide), GP (germination percentage), GR (germination rate), GU (germination uniformity), MGT (mean germination time.

Table 6
Analysis of variance of the effect of treatments on soybean seed antioxidant enzymes

SOV	Df	SOD	CAT	POX
SA	1	$^{**}1622.52$	$^{**}444.91$	$^{**}3520.30$
SNP	2	$^{**}3726.52$	$^{**}71.68$	$^{**}1487.63$
H_2O_2	2	$^{**}4075.24$	$^{**}353.46$	$^{**}1886.24$
SA × SNP	2	$^{**}42.74$	$^{**}9.80$	20.52^{*}
SA × H_2O_2	2	13.02^{ns}	2.35^{ns}	$^{**}26.35$
SNP × H_2O_2	4	$^{**}509.46$	3.57^{ns}	17.18^{*}
SA × SNP × H_2O_2	4	16.57^{ns}	4.91^{ns}	3.91^{ns}
Error	36	6.42	2.02	5.70
CV%		5.13	10.05	2.93

ns, *, and **, are non-significant, and significant at 0.05 and 0.01% probability levels, respectively.
SOD (superoxide dismutase), CAT (catalase), POX (peroxidase)

oxidative stress 60 µM of SNP is recommended to use in soybeans in order to have a better germination and seedling emergence.

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