SALICYLIC ACID ROLE ON SOYBEAN (*Glycine max* L.) GROWTH IN DROUGHT STRESS

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Abstract

Salicylic acid (SA) is an endogenous plant growth hormone responsive to drought stress. Therefore, this study was conducted to assess the exogenous SA impact on soybean (Glycine max L.) growth under drought stress conditions. An alcohol-carbohydrate, mannitol, at seven concentrations (0, 20, 25, 30, 35, 40, and 45 g/L) was used to determine the drought condition. The SA supplement in MS medium (0.5; 1.0; 1.5 and 2.0 mg/L) and the seed pretreatment with SA (2, 4, 6, and 8 mg/L) were used. Results showed that applied treatments significantly influenced soybean growth in drought stress condition. The supplement of 1.0 mg/L SA into medium and the seed pretreatment with 6.0 mg/L SA performed superior to improving the seedling growth under the drought stress. The SA presence not only improved the relative water content of leaves but also increased content of chlorophyll a and photosynthesis intensity.

Keywords: *Drought stress, Glycine max L., growth, salicylic acid, soybean.*

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VAI TRÒ CỦA SALICYLIC ACID LÊN SỰ TĂNG TRƯỞNG CỦA CÂY ĐẬU NÀNH (*Glycine max* L.) TRONG ĐIỀU KIỆN HẠN

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Tóm tắt

Salicylic acid (SA) là một chất điều hòa tăng trưởng thực vật nội sinh đóng vai trò quan trọng trong việc đáp ứng với stress hạn. Do đó, nghiên cứu này được tiến hành nhằm đánh giá tác động của SA ngoại sinh đến sự tăng trưởng của đậu nành (Glycine max L.) trong điều kiện khô hạn. Thí nghiệm sử dụng mannitol ở các thang nồng độ (0, 20, 25, 30, 35, 40 và 45 g/L) để xác định ngưỡng gây hạn của cây. Sau đó, việc bổ sung SA vào môi trường MS ở các thang nồng độ 0.5; 1.0; 1.5 và 2.0 mg/L và tiền xử lý hột với SA ở các thang nồng độ 2, 4, 6 và 8 mg/L được thực hiện. Kết quả nghiên cứu cho thấy rằng các xử lý SA ảnh hưởng đáng kể đến sự tăng trưởng của đậu tương trong điều kiện khô hạn. Việc bổ sung SA 1.0 mg/L vào môi trường nuôi cấy và tiền xử lý hột với SA 6 mg/L cho hiệu quả vượt trội trong việc cải thiện tốc độ tăng trưởng của cây. Kết quả cũng chỉ ra việc áp dụng SA 1.0 mg/L không những giúp gia tăng hàm lượng nước tương đối trong lá mà còn làm tăng hàm lượng chlorophyll a và cường độ quang hợp.

Từ khóa: *Stress hạn, Glycine max L., tăng trưởng, salicylic acid, đậu nành.*

1. Introduction

Soybean (*Glycine max* L.) is one of the important crop species with high nutritional and economic value (Jia *et al*., 2020). It is a rich source of protein (35 - 47 %), lipid (12.5 - 25.0%), and micronutrients (Opperman and Varia, 2011). It also improves soil quality for its ability of nitrogen-fixing (Hungria and Mendes, 2015). According to Ray *et al*. (2013), soybean production may be more difficult to obtain enough yields for future demands due to climate changes, especially drought. The lack of water led to a decrease in soybean growth, resulting in a 40%-yield reduction (Yan *et al*., 2020). Therefore, it is significant to improve the drought resistance of soybean. Salicylic acid (SA) is a plant growth regulator of the endogenous phenolic group, which regulates metabolism and stress responses (Hayat *et al*., 2010). Some publications show that exogenous SA treatments increase stress tolerance in cucumber and cowpea (Nguyen Thi Phuong Dung *et al*., 2016 and 2017). In cucumber, spraying SA (0.25 mM or 0.50 mM) enhances the growth in artificial drought conditions (PEG-6000). In the drought stress, the growth improvement was expressed by agronomic parameters: shoot height, leaf number, leaf area, fresh weight, the content of chlorophyll and proline. Thus, the present study was conducted to assess the possible role of SA in improving drought tolerance in soybean.

2. Materials and methods

2.1. Materials

The soybean cultivars HL 203, obtained from the Institute of Agricultural Science For Southern Vietnam, were used as material.

2.2. Methods

2.2.1. Effects of mannitol on soybean growth

Soybean seeds were disinfected by ethanol 70^{o} for 5 minutes and inoculated into ½ MS medium. After germination, seeds were transferred to test tubes containing ½ MS medium with mannitol at different concentrations (20, 25, 30, 35, 40 and 45 g/L). After 4 weeks, shoot height, leaf number, leaf area, root length and fresh weight of plants were determined. Shoot height and root length were measured with a ruler. True leaf number was counted. Leaf area was immediately measured by LIA-32 software. Fresh weight was measured by electronic scale.

2.2.2. Effects of SA on soybean growth under drought stress

Germinated seeds were cultured into test tubes containing ½ MS medium with 30 g/L of mannitol and SA at four concentrations (0.5; 1.0; 1.5 and 2.0 mg/L). After 4 weeks, shoot height, leaf number, leaf area, root length and fresh weight of plants were determined.

2.2.3. Effects of SA pretreatment on soybean growth under drought stress

The soybean seeds were disinfected by ethanol 70o for 5 minutes, rinsed with sterilized water for 10 minutes. Then, seeds were pretreated with SA at four concentrations (2, 4, 6, and 8 mg/L) for 60 minutes and cultured into test tubes containing ½ MS medium with 30 g/L of mannitol. After 4 weeks, shoot height, leaf number, leaf area, root length and fresh weight of plants were determined.

2.2.4. Analysis of physiological and biochemical changes

The relative water content of leaves (RWC) was determined based on the difference between fresh weight, water saturation, and dry weight (Sade *et al*., 2015). The leaf samples were immediately weighed and were placed in a water-containing plastic tube to record turgid weight. Then, leaves were oven-dried at 70^{o}C to determine dry weight.

The rate of photosynthesis were measured by oxygen electrode in the gas phase using the chamber of Leaflab2 (Hansetech, UK) in light condition (2000 lux).

The content of chlorophyll a and b: Leaf samples were ground in 95% ethanol, centrifuged at 3,000 rpm for 10 min to take the supernatant. The absorption was measured at 470 and 648 nm using a UV-VIS spectrophotometer (Lichtenhaler, 1987).

The total sugar and starch content was extracted using 80% ethanol, centrifuged at 3,000 rpm for 10 minutes to take the supernatant. This supernatant was mix with phenol-sulfuric acid and measured at 490 nm. The residue was dried, hydrolyzed by HClO4, centrifuged to take the supernatant. This supernatant was used to determine starch content by rapid colorimetric method with phenol-sulfuric acid (Coombs *et al*., 1987).

Proline content was determined according to the

method described by Paquin and Lechasseur (1979). Leaf samples were ground, homogenized with 5 mL ethanol, and centrifuged at 3000 rpm for 5 minutes. The extract was mixed with glacial acetic acid and ninhydrin reagent for 1 hour at 100°C, and cool at room temperature. Readings were taken immediately at a wavelength of 520 nm. The proline concentration was determined from a standard curve.

2.2.5. Experimental design and statistical analysis

Experiments were carried out under light intensity of 2000 ± 200 lux (12/24 hours), 27 ± 2°C, and humidity 58 ± 5%. The experiments were carried based on randomized complete blocks with three replications. All results were presented as means and standard deviation. Statistical assays were carried out by one-way ANOVA using Duncan posthoc-test with significant differences at a 5% level of probability.

3. Results and discussion

3.1. Effects of mannitol on soybean growth

Table 1. Effects of mannitol on growth of soybean seedling after 28 planting days

Mannitol (g/L)	Shoot height (cm)	Leaf number	Leaf area (cm^2)	Root length (cm)	Fresh weight (g)
0 (Control)	12.50 ± 0.18^a	2.78 ± 0.38^a	41.29 ± 0.42^a	5.63 ± 0.15^a	1.72 ± 0.10^a
20	11.76 ± 0.28^b	2.56 ± 0.19^a	36.51 ± 0.81^b	5.26 ± 0.13^b	1.42 ± 0.03^b
25	10.59 ± 0.42^c	2.11 ± 0.19^b	22.71 ± 0.57^c	5.03 ± 0.20^b	1.26 ± 0.07^c
30	10.38 ± 0.50^c	2.00 ± 0.00^b	20.78 ± 0.63^d	4.28 ± 0.08^c	1.10 ± 0.01^d
35	5.46 ± 0.05^d	0.00 ± 0.00^c	0.00 ± 0.00^e	2.29 ± 0.07^d	0.98 ± 0.02^e
40	-	-	-	-	-
45	-	-	-	-	-

Note: (-) dead seeling; In each column, means with different letters are significantly different according to Duncan's test (p=0.05).

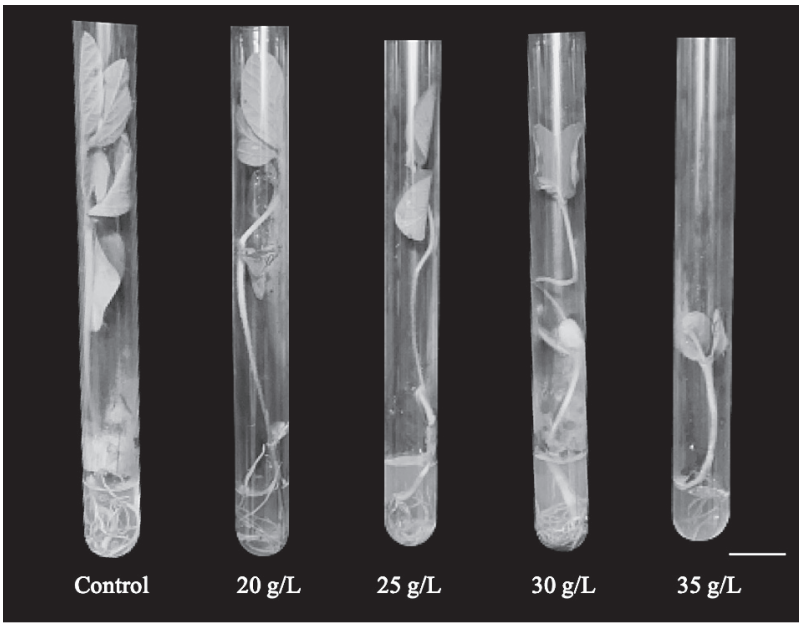

Figure 1. Cultured soybean seedling in media of different mannitol concentrations after 28 culture days. Bar 2 cm

The results of the experiment presented in Table 1 showed that mannitol with a concentration of 25 g/L caused drought stress on soybean. The increase in mannitol concentration led to a decrease in soybean growth. After 28 planting days in ½ MS medium with 30 g/L mannitol, leaves not only wilted but also reduced approximately 50% the total area compared to the control (Figure 1). This experiment showed that soybean adapted well to mannitol at 20 g/L or under.

Mannitol is an osmotic adjustment factor decreasing water potential outside the cells. As a result, cells cannot absorb sufficient water for normal functions. According to a publication by Kulpa *et al*. (2018), soybean seeds were caused drought stress when these were grown on the media containing 200 mM of mannitol (36.4 g/L). Seedlings had leaf number, leaf area, and biomass lower than the control plants. For French beans (*Phaseolus vulgaris*), mannitol concentration at 150 mM (27 g/L) inhibited the length of the root and biomass (Aydi *et al*. (2008). It showed that the effect of mannitol is dependent on the genotype of species.

3.2. Effects of SA on soybean growth under drought stress

The effect of SA on soybean growth under drought stress was shown in Table 2. After 28 days of planting on ½ MS with 30 g/L mannitol, the application of 1.0 mg/L SA gave better results than other SA concentrations. ½ MS medium supplemented with 1.0 mg/L SA resulted in the highest leaf number (3.00), leaf area (33.78 cm^2) and fresh weight (1.50 g). There was no difference in the number of leaves and the length of root among the treatments (Figure 2). It matched with study results of Antonić *et al*. (2020), who indicated that the supplement SA in culture medium improved the development of *Impatiens walleriana* in the drought stress. SA may modulate the responses to drought stress by inducing the expression of PR1 and PR2 (Miura *et al*., 2013). Also, SA increased the membrane stability, ABA level, and stimulated the ascorbate-glutathione cycle in barley (Bandurska and Stroinski, 2005; Kang *et al*., 2013).

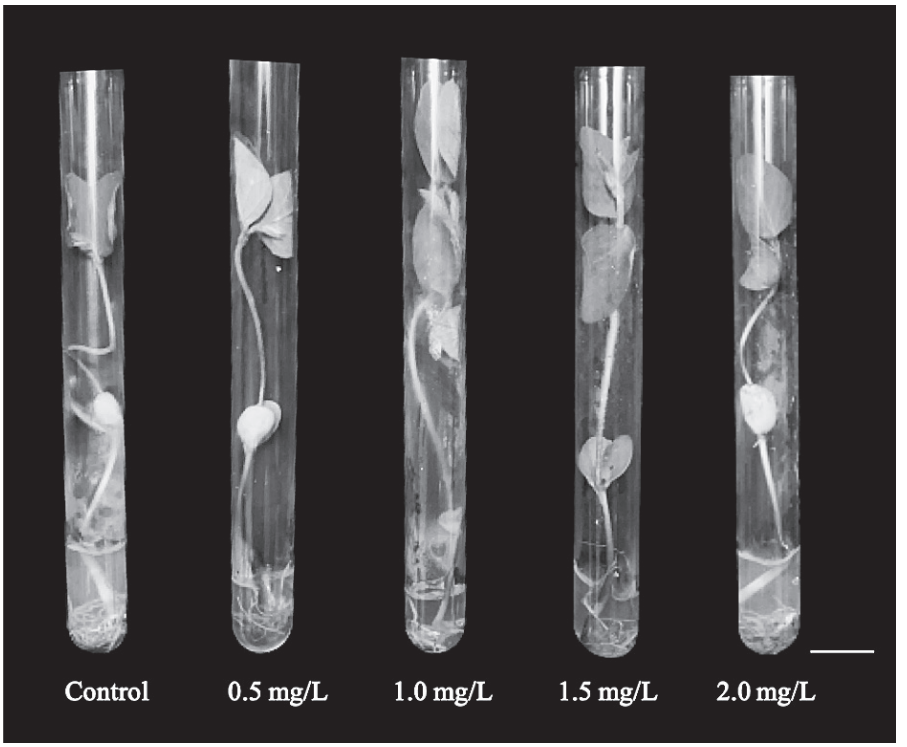

Figure 2. Cultured soybean seedling in media of 30 g/L mannitol and different SA concentrations after 28 planting days. Bar 2 cm

Table 2. Effects of SA on growth of soybean in the drought stress condition after 28 days

SA (mg/L)	Shoot height (cm)	Leaf number	Leaf area (cm^2)	Root length (cm)	Fresh weight (g)
0 (Control)	10.38 ± 0.50^{b}	2.00 ± 0.00^{b}	20.78 ± 0.63^{d}	4.28 ± 0.08^{c}	1.09 ± 0.01^{c}
0.5	10.18 ± 0.16^{b}	2.00 ± 0.00^{b}	23.09 ± 0.07^{c}	4.36 ± 0.07^{bc}	1.21 ± 0.01^{b}
1.0	12.73 ± 0.07^{a}	3.00 ± 0.00^{a}	33.78 ± 0.81^{a}	4.16 ± 0.04^{d}	1.50 ± 0.01^{a}
1.5	12.26 ± 0.25^{a}	2.00 ± 0.00^{b}	25.2 ± 0.15^{b}	5.44 ± 0.07^{a}	1.26 ± 0.06^{b}
2.0	10.53 ± 0.44^{b}	2.00 ± 0.00^{b}	24.4 ± 0.13^{b}	4.44 ± 0.07^{b}	1.23 ± 0.04^{b}

Note: In each column, means with different letters are significantly different according to Duncan's test (p=0.05).

3.3. Effects of SA pretreatment on soybean growth under drought stress

The achieved results of the experiment are displayed in Table 3. After 28 planting days, the seedling growth of 6 mg/L SA pre-treatment was significantly effective compared to the other treatments. The height of shoot, the number of leaves, the total leaf area, and fresh weight were higher than others. Figure 3 shows that seedlings growed well with green leaves. Many previous studies showed the positive effect of SA treatment in maize (Saruhan *et al*., 2012), common bean (Sadeghipour and Aghaei, 2012), and wheat (Sharafizad *et al*., 2013).

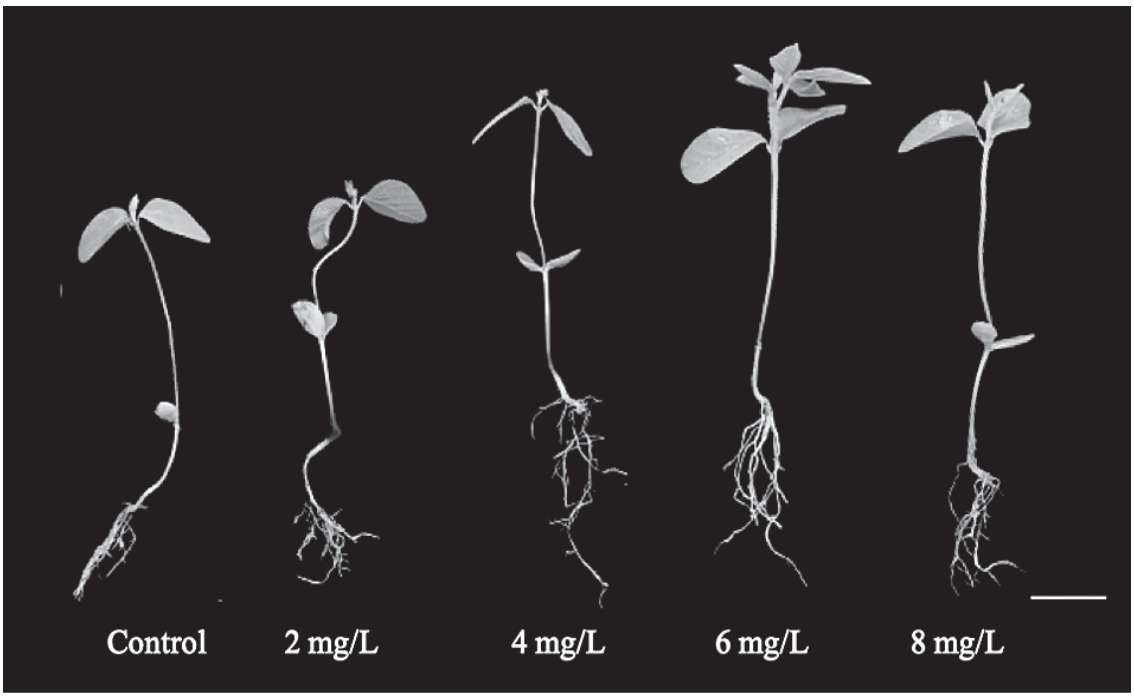

Figure 3. Effects of SA pretreatment on soybean growth in drought stress condition after 28 culture days. Bar 3 cm

Table 3. Effects of SA pretreatment on growth of soybean seedling in the drought stress condition after 28 planting days

SA (mg/L)	Shoot height (cm)	Leaf number	Leaf area (cm^2)	Root length (cm)	Fresh weight (g)
0 (Control)	10.38 ± 0.50^{b}	2.00 ± 0.00^{b}	20.78 ± 0.63^{d}	4.28 ± 0.08^{d}	1.10 ± 0.01^{d}
2	10.38 ± 0.28^{b}	2.00 ± 0.00^{b}	23.97 ± 0.71^{c}	5.13 ± 0.10^{c}	1.22 ± 0.01^{c}
4	10.46 ± 0.05^{b}	2.00 ± 0.00^{b}	24.26 ± 0.17^{c}	7.30 ± 0.07^{a}	1.28 ± 0.02^{b}
6	12.21 ± 0.08^{a}	3.00 ± 0.00^{a}	35.01 ± 0.75^{a}	7.09 ± 0.12^{b}	1.43 ± 0.03^{a}
8	11.91 ± 0.13^{a}	2.00 ± 0.00^{b}	25.49 ± 0.86^{b}	5.10 ± 0.10^{c}	1.28 ± 0.01b

Note: In each column, means with different letters are significantly different according to Duncan's test (p=0.05).

3.4. Effects of SA on physiology and biochemical changes in drought stress

Under drought stress, the growth-promoting effects of exogenous SA could be related to changes in RWC, photosynthesis, pigments, and carbohydrate metabolism. The results from table 4 showed that the photosynthesis, RWC, starch content, and the content of chlorophyll *a* decreased strongly in the drought stress condition but increased in the case of 1 mg/L SA pretreatment. In contrast, more proline and total sugar were significantly enriched under the drought stress condition compared to the control but the accumulation of proline and sugar decreased in seedlings treated with SA. Under drought stress, the increase in soluble sugar not only provides energy substrate but also regulates water potential and signaling in cells. Similarly, the enhanced proline synthesis also contributes to the balance of water. Moreover, proline maintains the stabilization of proteins, antioxidant enzymes, and balance of intracellular redox homeostasis (Liang *et al*., 2013).

The study by Sharma *et al*. (2018) was performed in soybean under severe and moderate drought regimes demonstrated that SA stimulated the carbon and nitrogen metabolism, and biosynthesis of proteins which involved in photosynthesis and energy. This helps to drive growth and stress adaptation. Low SA concentration may be increased Rubisco activity and chlorophyll content but it can cause a decrease in photosynthesis at a high concentration (Yusuf *et al*., 2013). In this study, the 1 mg/L SA pretreatment improves relative water content and chlorophyll a content, and this leads to an increase in CO2 assimilation and starch content in leaves (Table 4). According to a publication by Chen *et al*. (2014), the pretreatments of zoysiagrass seeds with 0.1 and 0.5 mM SA significantly increased fresh and dry weights and chlorophyll content, while 1 mM SA pretreatment did not show significant change compared to controls under drought stress. This shows the beneficial effects of SA depending on plant species and applied SA concentration. In soybean, the seed pretreatment with the low dose of SA (1 mg/L) induced growth improvement under drought stress.

Table 4. Effects of SA pretreatment on physiology and biochemical changes in the drought stress condition after 28 planting days

Treatments	The control (without manntiol)	Drought stress (30 g/L mannitol)	1.0 mg/L SA
RWC (%)	92.51 ± 0.17[a]	61.94 ± 0.31[c]	87.99 ± 1.79[b]
Photosynthesis ($\mu M\ O_2/cm^2/h$)	53.31 ± 0.39[a]	25.38 ± 1.41[c]	45.94 ± 1.41[b]
Chlorophyll a (mg/g FW)	0.91 ± 0.01[a]	0.66 ± 0.01[c]	0.82 ± 0.01[b]
Chlorophyll b (mg/g FW)	0.35 ± 0.02[a]	0.35 ± 0.02[a]	0.35 ± 0.05[a]
Total sugar (mg/g FW)	15.14 ± 0.24[b]	22.58 ± 0.25[a]	15.71 ± 0.36[b]
Starch (mg/g FW)	37.44 ± 1.26[a]	23.37 ± 0.94[c]	33.22 ± 1.69[b]
Proline (mg/g FW)	1.47 ± 0.07[c]	4.87 ± 0.20[a]	2.52 ± 0.12[b]

Note: In each row, means with different letters are significantly different according to Duncan's test (p=0.05).

4. Conclusions

In the *in vitro* condition, soybean was drought-stressed at the concentration of mannitol 25 $g.L^{-1}$. Under drought stress, the relative water content of leaf, photosynthesis, starch content, and chlorophyll a content decreased but proline and sugar content increased.

The supplement of 1.0 mg/L SA into medium or the seed pretreatment with 6 mg/L SA showed positive results for the growth of soybean. The application of SA led to an increase in RWC, chlorophyll a content and photosynthesis compared to the drought stress.

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