

Effect of foliar application of chitosan and salicylic acid on the growth of soybean (*Glycine max* (L.) Merr.) varieties

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Abstract. Elicitors such as chitosan and salicylic acid could be used not only to increase isoflavone concentration of soybean seeds, but also to increase the growth and seed yield. The objective of the present study was to determine the effects of foliar application of elicitor compounds (i.e. chitosan, and salicylic acid) on the growth of two soybean varieties under dry land conditions. Experimental design was a randomized block design with 2 factors and 3 replications. The first factor was soybean varieties (Wilis and Devon). The second factor was foliar application of elicitors consisted of without elicitor; chitosan at V4 (four trifoliate leaves are fully developed); chitosan at R3 (early podding); chitosan at V4 and R3; salicylic acid at V4; salicylic acid at R3 and salicylic acid at V4 and R3. Parameters observed were plant height at 2-7 week after planting (WAP), shoot dry weight and root dry weight. The results suggest that the Wilis variety had higher plant height 7 WAP than Devon. The foliar application of chitosan increased the plant height at 7 WAP, shoot dry weight and root dry weight. The foliar application of chitosan at V4 and R3 on Devon variety increased shoot dry weight.

1. Introduction

Interest in soybeans has greatly increased in recent years because of the positive effects of soybean on human health, particularly due to isoflavone content in soybean. Isoflavones as a secondary metabolite compound in soybean plants are phytoestrogens that act as antioxidants for chemopreventive agents and estrogenic compounds that can inhibit cell proliferation, as well as to prevent osteoporosis and heart damage, reduce menopausal syndrome and anti-tumor to prevent degenerative diseases such as arteriosclerosis, prostate cancer and colon cancer and diabetes mellitus [1, 2, 3, 4].

The accumulation of isoflavones in soybean is determined by genetic (internal) and environmental (external) factors. Internal factors that affect the accumulation of isoflavones are related to gene activity. Influencing environmental factors consist of biotic factor, such as wounding, nodulation and pathogen attack, and abiotic elements such as temperature, water regime, UV light, soil nutrient content, carbon dioxide and also elicitor [5, 6, 7, 8, 9, 10, 11, 12].

Increased accumulation of isoflavones in soybeans can be done by inducing soybeans with biotic or abiotic elicitors that will stimulate the formation of phytoalexin in soybeans. Elicitation is an efficient strategy that by means of compounds or treatments induces plants to synthesize phytoalexins at enhanced levels [13]. Several studies have shown that the elicitation method may increase the phytoalexin content and secondary metabolites in soybean crops. The usefulness of elicitors is as a strategy in inducing and promoting the formation of secondary metabolites and can increase the activity of specific enzymes associated with the formation of secondary metabolites [14]. Previously



research by author reported that the treatment of chitosan and salicylic acid elicitors in the screen house experiments resulted in the highest levels of genistein, daidzein and isoflavone content compared to other treatments [15].

Elicitors such as chitosan and salicylic acid could be used not only to increase isoflavone concentration of soybean seeds, but also to increase the growth and seed yield. Several studies carried out under laboratory or field conditions strongly suggest that salicylic acid play an important role in many biological responses in plants. The effect of these substances on the physiology of the plants is variable, promoting some processes and inhibiting others [16]. Salicylic acid and its regulatory role in plant physiology include inhibiting ethylene biosynthesis, interfering with membrane depolarisation, blocking wound responses, and an increase in photosynthetic rate and chlorophyll content in soybeans [17]. The results from the literature show that, chitosan can increase the yield when used in plants, [18], reduce transpiration [19] and induce a range of metabolic changes as a result of which, plants become more resistant to viral, bacterial and fungal infections [20].

Based on the background, the objective of the present study was to determine the effect of foliar application of elicitor compounds (i.e. chitosan, and salicylic acid) on the growth of two soybean varieties under dry land conditions.

2. Materials And Methods

2.1. Study area

The field experiment was conducted at the field grown of Medan Selayang, Medan (Indonesia). The soil content of Nitrogen was low (0.26%), organic matter was 1.02%, with a pH of 4.5.

2.2. Procedures

Treatments were arranged in a Randomized Block Design with two factors and three replications. The first factor was soybean varieties (Wilis and Devon). The second factor was foliar application of elicitors consisted of without elicitor ; chitosan at V4 (four trifoliate leaves are fully developed) ; chitosan at R3 (early podding) ; chitosan at V4 and R3 ; salicylic acid at V4 ; salicylic acid at R3 and salicylic acid at V4 and R3. Preparation of elicitor referred to standard procedures. Chitosan and salicylic acid is a product of Sigma Aldrich. Autoclaved stock solution of 120°C for 20 minutes, and sterile distilled water to obtain a final concentration of chitosan solution 0.5 mg/mL. Salicylic acid dissolved in distilled water and diluted to concentrations (0.5 mM). The determination of the concentration of salicylic acid refers to previous research by Al Hetar *et. al* [21].

Soybean seeds that have been inoculated by *Bradyrhizobium japonicum* are planted with plant spacing 40 cm x 20 cm. N fertilizer application at dose of 50 kg ha⁻¹ Urea was given half the dose of N fertilizer at planting time and the rest at 4 WAP. At planting time, phosphor and potassium fertilizer application at a dose of 150 kg ha⁻¹ P₂O₅ and 75 kg K₂O ha⁻¹ for all soybean crops. Weeding was done manually by removing the weeds in accordance with the conditions of the field.

Parameters observed was plant height at 2-7 week after planting (WAP), shoot dry weight and root dry weight.

2.3. Data analysis

Data were subjected to analysis of variance (ANOVA) for comparison of means. Means were separated using Duncan's Multiple Range Test at the 0.05 probability level.

3. Results and Discussion

3.1. Plant height

Based on Table 1. it can be seen that the treatment of varieties has significant effect on plant height at 2, 3, 4 and 7 WAP. The elicitor treatment had significant effect on plant height at 4 WAP. Devon variety have plant height at 2-4 WAP that is significantly higher than Wilis, while at plant height 7 WAP the Wilis variety significantly higher than Devon. It is assumed that the difference in plant height between varieties is due to plant genetic factors and is associated with faster Devon plant

growth rate compared to Wilis at the beginning of the vegetative growth phase. At the age of 7 WAP, the combination of Wilis and elicitor of chitosan at R3 increased plant height. This correlated with increases in stomatal conductance and transpiration rate. Chitosan foliar application did not have any effect on the intercellular CO₂ concentration. Previously authors reported that the observed effect on the net photosynthetic rate is, in general, common in maize and soybean after foliar application of high molecular weight chitosan [22]

Table 1. Effect of foliar application of chitosan and salicylic acid on plant height 2-7 WAP of two soybean varieties

soybean varieties		Elicitor							Mean
WAP	Variety	E0	E1	E2	E3	E4	E5	E6	
	cm.....							
2	V1 (Wilis)	9.28	8.83	9.25	9.49	9.02	9.35	8.55	9.11b
	V2 (Devon)	13.18	12.39	11.87	12.88	12.17	11.49	12.20	12.31a
	Mean	11.23	10.61	10.56	11.19	10.59	10.42	10.37	
3	V1 (Wilis)	12.94	12.14	14.06	13.16	13.00	13.50	13.13	13.13b
	V2 (Devon)	16.19	17.09	16.86	17.34	16.92	15.25	17.01	16.67a
	Mean	14.57	14.61	15.46	15.25	14.96	14.38	15.07	
4	V1 (Wilis)	25.67	21.62	28.20	25.37	25.90	23.73	25.40	25.13b
	V2 (Devon)	26.87	28.43	29.22	29.33	28.58	24.20	29.47	28.01a
	Mean	26.27cd	25.03de	28.71a	27.35bc	27.24bc	23.97e	27.43b	
5	V1 (Wilis)	34.37	30.40	37.83	34.97	34.87	35.07	34.70	34.60
	V2 (Devon)	35.43	36.69	38.67	36.92	38.77	32.00	35.07	36.22
	Mean	34.90	33.54	38.25	35.94	36.82	33.53	34.88	
6	V1 (Wilis)	52.13	44.80	54.93	51.73	49.53	48.53	52.10	50.54
	V2 (Devon)	47.20	49.83	52.73	51.10	54.10	45.07	50.67	50.10
	Mean	49.67	47.32	53.83	51.42	51.82	46.80	51.38	
7	V1 (Wilis)	65.17	55.82	67.99	64.93	61.03	61.11	63.17	62.74a
	V2 (Devon)	55.64	58.83	61.69	59.16	60.10	53.69	57.56	58.09b
	Mean	60.40	57.32	64.84	62.04	60.56	57.40	60.36	

Note : E0 =withoutelicitor ; E1 = Chitosan at V4 ; E2 = Chitosan at R3 ; E3 = Chitosan at V4 dan R3 ; E4 = Salicylicacid at V4 ; E6 = Salicylicacid at R3 dan E6 =Salicylicacid at V4 dan R3. Means followed by the same letters at the same coloum, row and time of absoervation not significantly differ base on Duncan's Multiple Range test (p=0.05). This note also used for Table 2 and 3.

3. 2. Root dry weight

The result showed that Devon variety have higher root dry weight than Wilis. The chitosan treatment on V4 (E2) yielded higher root dry weight than other treatments. The combination of Devon treatment and chitosan elicitor on V4 increased root dry weight (Table 2). This suggests that chitosan plays a role in increasing root growth which is indicated by increasing root dry weight. Foliar application of chitosan at V4 increases root dry weight. In line with previously research reported that foliar application of chitosan stimulates increased vegetative growth of plants and protects plants against oxidative stress [23, 24, 25].

Table 2. Effect of foliar application of elicitor (chitosan and salicylic acid) on root dry weight of two soybean varieties

soybean varieties								Mean
Variety	Elicitor							
	E0	E1	E2	E3	E4	E5	E6	
	g							
V1 (Wilis)	1.76	1.11	1.70	1.51	1.68	1.49	1.39	1.52
V2 (Devon)	1.70	1.60	2.09	1.48	1.37	1.20	1.54	1.57
Mean	1.73	1.36	1.89	1.49	1.53	1.35	1.46	

3. 3. Shoot dry weight

Devon varieties have higher shoot dry weight than Wilis. The chitosan treatment on V4 (E3) yielded higher shoot dry weight than other treatments. The combination of Devon treatment and chitosan elicitor on V4 increased shoot dry weight (Table 3). This suggests each variety has different growth rates and chitosan application on V4 enhances the growth rate that indicated with increased shoot dry weight. Previously research reported that chitosan play a positive role in plant growth associated with its effects in increased nutrient uptake such as N, P and K which play an important role in the biosynthesis and translocation of carbohydrates and stimulates cell division, cell turgor and DNA and RNA formation [26]. The other authors reported that the increase in *Phaseolus* yield due to chitosan application may be due to its effects in stimulating physiological processes, improving vegetative growth, followed by active translocation of photoassimilates from source to sink tissues. The increases in plant biomass may be due to improving photosynthetic machinery, and chitosan promoted the plant and root growth have suggested that the growth promotion might be a nitrogen effect because chitosan contains about 8.7% N [27]

Table 3. Effect of foliar application of elicitor (chitosan and salicylic acid) on shoot dry weight of two soybean varieties

Variety	Elicitor							Mean
	E0	E1	E2	E3	E4	E5	E6	
g.....							
V1 (Wilis)	13.47	3.63	13.37	9.52	15.19	11.02	8.72	10.70
V2 (Devon)	11.74	12.79	7.61	17.55	8.71	10.03	10.19	11.23
Mean	12.61	8.21	10.49	13.53	11.95	10.53	9.46	

4. Conclusion

Wilis variety had higher plant height 7 WAP than Devon. The foliar application of chitosan increased the plant height at 7 WAP, shoot dry weight and root dry weight. The foliar application of chitosan at V4 and R3 on Devon variety increased shoot dry weight.

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