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 was 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 effects of foliar application of elicitor compounds (i.e. chitosan, and salicylic acid) on the growth of two soybean varieties under dryland 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

| Variety | E0 | E1 | E2 | E3 | E4 | E5 | E6 | Mean |
|---------|----|----|----|----|----|----|----|------|
| 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 | |
| V1 (Wilis) | 12.94 | 12.14 | 14.06 | 1 | 3.16 | 13.00 | 13.50 | 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 | |
| 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 | 28.58 | 24.20 | 17.01 | 16.67a | |
| Mean | 26.27 | 25.03 | 28.71 | 27.35 | 27.24 | 23.97 | 27.43 | |
| V1 (Wilis) | 34.37 | 30.40 | 37.83 | 34.97 | 34.87 | 35.07 | 34.70 | 36.22 |
| V2 (Devon) | 35.43 | 36.69 | 38.67 | 36.92 | 38.77 | 32.00 | 35.07 | |
| Mean | 34.90 | 35.54 | 38.25 | 35.94 | 36.82 | 33.53 | 34.88 | |
| 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 ; E5 =Salicylicacid at V4 dan R3. Means followed by the same letters at the same coloum, row and time of abservation 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

| Variety | E0 | E1 | E2 | E3 | E4 | E5 | E6 | Mean |
|---------|----|----|----|----|----|----|----|------|
| 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 | E0   | E1   | E2   | E3   | E4   | E5   | E6   | Mean  |
|----------|----------|------|------|------|------|------|------|------|-------|
| 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.

References
[1] Barnes S 2001 Soy isoflavones and cancer p. 49–53. In: K. Descheemaeker and I. Debruyn (ed.) Soy and health. Garant. Leuven. Belgium.
[2] Bennett JO, OYu, LG Heatherly and HB Krishnan 2004 Accumulation of genistein and daidzein soybean isoflavones implicated in promoting human health. is significantly elevated by irrigation. J Agric. Food Chem. 52: 7574 – 7579.
[3] Shinde AN, N Malpathak, DP Fulzele2009 Enhanced production of phytoestrogenic isoflavones from hairy root cultures of *Psoralea corylifolia* L. using elicitation and precursor feeding Biotechnology and Bioprocess Engineering 14, 288–294.
[4] Tsukamoto C, Shimada S, Irita K, Kudou S, Kokubun M, Okubo K and Kitamura K 1995 Factors affecting isoflavones content in soybean seeds: changes in isoflavones, saponins, and composition of fatty acids at different temperatures during seed development J. Agric. Food. Chem. 43: 1184 -1192.
[5] Nelson RL, Lozovaya V, Lygin A and Widholm J 2001 Variation in isoflavones in seeds of domestic and exotic soybean germplasma In: 2001 Agronomy Abstracts (CD-ROM). ASA. CSSA and SSSA. Madison. WI.
[6] Ososki ALand Kennelly EJ2003. Phytoestrogens: a review of the present state of research. Phytother. Res. 17: 845 - 869.
[7] Vyn T J, Yin X, Bruulsema TW, Jackson CC, Rajcan I and Brouder SM 2002 Potassium fertilization effects on isoflavone concentrations in soybean (*Glycine max* (L.) Merr.) J. Agric. Food. Chem. 50: 3501–3506.
[8] Lozovaya VV, Lygin AV, Ulanov AV, Nelson RL, Dayde J and Widholm AM 2005 Effect of temperature and soil moisture status during seed development on soybean seed isoflavone concentration and composition. Crop Sci. 45:1934–1940.

[9] Subramanian S, G Stacey, O Yu 2005 Endogenous isoflavones are essential for the establishment of symbiosis between soybean and *Bradyrhizobium japonicum*. Plant J. 48:261–273.

[10] Zhang B Hettiarachchy N Chen PHorax R CorneliusB ZhuD 2006 Influence of *The Application of Three Different Elicitors on Soybean Plants on The Concentrations of Several Isoflavones in Soybean Seeds*. Journal Of Agricultural and Food Chemistry 54, 5548–5554.

[11] Naoumkina M Farag MA Sumner LW Tang Y Liu CJand Dixon RA 2007 Different mechanisms for phytoalexin induction by pathogen and wound signals in *Medicago truncatula*. Proc. Natl Acad. Sci. USA 104:17909–17915.

[12] Phommalth S JeongYS Kim YH Dhakal KH Hwang YH 2008 Effects of light treatment on isoflavone content of germinated soybean seeds. Journal of Agriculture and Food Chemistry 56, 10123–10128.

[13] Gonzalez JJ SK Guttikonda LS Phan Tran DL Aldritch R Zhong OYu HT Nguyen and DA Sleper 2010Differential expression of isoflavone biosynthetic genes in soybean during water deficits. Plant and Cell Physiol. 51(6):936-948.

[14] Rao RS Ravishankar GA 2002 Plant Cell Cultures: Chemical Factories Of Secondary Metabolites. Biotechnology Advances 20, 101–153.

[15] Roberts SC 2005 Plant metabolic engineering for pharmaceutical production. www. metabolic engineering.gov/me2005/Roberts.pdf.

[16] Hasanah Y, LAM Siregar, L Mawarni 2016 Final Report of Competitive Grant Research Year "The Role of Elicitors in Increasing Soybean Isoflavones Content as the Functional Food". Universitas Sumatera Utara. Medan.

[17] Raskin I 1992 Role of salicylic acid in plants, Annu. Rev. Plant Physiol. Plant Mol. Biol. 43:439-463.

[18] Leslie CA, Romani RJ 1988 Inhibition of ethylene biosynthesis by salicylic acid, Plant Physiol. 88:833–837.

[19] Mondal MMA, Malek MA, Puteh AB, Ismail MR, Ashrafullazaman M, Naher L 2012 Effect of foliar application of chitosan on growth and yield in okra. Aust. J. Crop. 6:918-921.

[20] Dzung NA, Khanh VTP, Dzung TT 2011 Research on impact of chitosan oligomers on biophysical characteristics, growth, development and drought resistance of coffee. Carbohydr. Polym., 84:751-755.

[21] Al-Hetar MY, Zainal Abidin MA, Sariah M, Wong MY 2011 Antifungal activity of chitosan against Fusarium oxysporum f. sp. cubense J. Appl. Polym. Sci., 120:2434-2439.

[22] Saini RK, M Akithadevi, Parvatamgiridhar, G Ravishankar 2013 Augmentation of major isoflavones in *Glycine max* L. Acta Bot. Croat. 72(2), 311–322.

[23] El Hadrami ALR Adam I El Hadrami and D Daayf 2010 Chitosan in Plant Protection Mar Drugs 8(4):968-987.

[24] Guan Ya-jing, Jin Hu, Xian-ju Wang, Chen-xia Shao 2009 Seed priming with chitosan improves maize germination and seedling growth in relation to physiological changes under low temperature stress J. of Zhejiang University Science B 10(6):427-433.

[25] Farouk S Mosa AA Taha AA Ibrahim Heba M EL-Gahmery AM 2011 Protective effect of humic acid and chitosan on radish (Raphanus sativus L. var. sativus) plants subjected to cadmium stress. Journal of Stress Physiology and Biochemistry 7(2):99-116.

[26] Ghoname AA EL-Nemr MA Abdel-Mawgoud AMR El-Tohamy WA 2010 Enhancement of sweet pepper crop growth and production by application of biological, organic and nutritional solutions. Research Journal of Agriculture and Biological Science 6(7):349-355.

[27] Farouk S and AR Amany 2012 Improving growth and yield of cowpea by foliar application of chitosan under water stress Egyptian J. of Biology 14:14-26.
[28] Khan MH, Singha KLB, Panda SK 2002 Changes in antioxidant levels in *Oryza sativa* L. roots subjected to NaCl salinity stress Acta Physiol. Plantarum 24:145–148.

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