

## Conference Paper

# Seed Characteristics of Soybean Cultivars As Affected by Foliar Application of Elicitors

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**Abstract**

The objective of the research was to determine seed characteristics of soybean cultivars on the foliar application of elicitors. The research used a randomized block design with three factors and three replications. The first factor was soybean cultivars (Anjasmoro and Wilis). The second factor was the foliar application of elicitors consisted of without elicitor; chitosan ( $1 \text{ mg} \cdot \text{mL}^{-1}$ ), methyl jasmonate (0.5 mM) and salicylic acid (0.5 mM). The third factor was the time of foliar application of elicitors consisted of V4 and R3. The results suggest that the response of each cultivar to the treatment type and timing of elicitor application is different. In Anjasmoro cultivar, the elicitor treatment of salicylic acid at R3 tend to produce the highest number of the filled pod (58.88 pod), while in Wilis cultivar, the elicitor treatment of methyl jasmonate at V4 tend to produce the highest number of the filled pod (73.63 pod). The yield response of dry weight of 100 seeds was observed with the application of chitosan at the R3 stage.

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## 1. Introduction

People's interest in soybeans significantly increased sharply in recent years because soybean plays a role for human health. Currently, soybean not only serves as a food source of protein, but it is highly potent in food security and also a source of functional food for human health. Soybean products contain organic compounds related to the isoflavone which act as phytoestrogen [1]. They have antiestrogenic, antioxidant, anti-inflammatory activities and are also associated with lower incidence of chronic diseases such as cancers, heart and kidney diseases and in addition, prevent bone loss [2–4]. Estrogenic and anti-estrogenic effects of isoflavone depend on the natural levels of estrogen. At a low level of natural estrogen, isoflavones mimic estrogen by activating the estrogen receptors [5], whereas, at high estrogen levels, they bind with estrogen receptors, decrease the availability of estrogen receptors and act as anti-estrogens [6].

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Isoflavone content in soybean seeds (and other soybean tissues) is influenced by biotic and abiotic factors including physical and chemical damages, UV light, low temperature, wounding, pathogens and plant-microbe interactions as well as elicitor treatment [7–10]. It is also found that these factors are involved in the up-regulation or induction of the phenylpropanoid pathway genes for the biosynthesis of isoflavones.

Increased accumulation of isoflavones in soybeans can be done by inducing a soybean with biotic or abiotic elicitor which stimulates the production of phytoalexin in soybean. Previously greenhouse studies have also demonstrated that exogenous applications of elicitor compounds including LCOs produced by rhizobia, chitosan (i.e., deacylated chitin), yeast extract, and actinomycete spores promote accumulation of isoflavone in soybean seeds [11].

The objective of the present study was to determine the effects of foliar applications of elicitor compounds (i.e., methyl jasmonate, salicylic acid, and chitosan) on the seed characteristics including the yield of soybean.

## 2. Materials and Methods

The research was conducted in Kassa house of Faculty of Agriculture University of Sumatera Utara (Indonesia) from March to June 2016. The soil of content of Nitrogen was low (0.14 %), organic matter was 1.02 %, with a pH of 4.5.

### 2.1. Experimental design and crop management

Treatments were arranged in a Randomized Block Design with three factors and three replications. The first factor was two soybean cultivars (Anjasmoro and Wilis). Selection of the cultivar referring to previously experiment conducted by Hasanah, et al. [12]. The second factor was elicitor sources and consisted of no elicitor; chitosan; methyl jasmonate and salicylic acid. The third factor was a time of application (V4 and R3). Selection of the type and concentration of elicitor referring to previous experiments conducted by Al Tawaha, et al. [11] and Sindhe, et al. [13]. Preparation of elicitor referred to standard procedures chitosan, methyl jasmonate and salicylic acid is a product of Sigma Aldrich. Chitosan is made from the shells of crabs using a procedure previously developed by Benhamou, et al., [14]. Autoclaved stock solution of 120 °C for 20 min, and sterile distilled water to obtain a final concentration of chitosan solution 1 mg · mL<sup>-1</sup>. Abiotic elicitor, salicylic acid and methyl jasmonate dissolved in distilled water and diluted to concentrations (0.5 mM). The determination of the concentration of methyl

jasmonate and salicylic acid refers to research Saini, et al. [15]. Parameters observed was number of pods, number of fulfilled pods, and seed dry weight per plant and dry weight of 100 seeds.

## 2.2. Statistical data analysis

Data were subjected to analysis of variance (ANOVA) for comparison of means. A combined analysis of variance was done to evaluate isoflavones affected by growing season. Means were separated using Duncan's Multiple Range Test at the 0.05 probability level.

# 3. Results and Discussion

## 3.1. Seed dry weight per plant

Mean comparisons for dry seed weight of soybean cultivars with different type and application time of elicitor are shown in Table 1. Cultivar Wilis is significantly higher seed dry weight per plant (10.94 g) than Anjasmoro (9.24 g). Anjasmoro cultivar with elicitor application on V4 produced the lowest seed dry weight per plant (8.66 g), while Wilis cultivar with elicitor application on the V4 produced the highest seed dry weight per plant (12.02 g). Time of application of elicitor V4 or R3 on the cultivars Wilis and Anjasmoro produced dry seed weight per plant were not significantly different, respectively 9.86 g and 9.83 g (Table 1).

Salicylic acid treatments tend to produce the highest seed dry weight. In Anjasmoro cultivar, the elicitor treatment of salicylic acid at V4 tend to produce the highest seed dry weight per plant (11.47 g). It is presumably related to the role of salicylic acid in increasing plant growth, photosynthesis and plant defense against pathogens that result to increase the dry weight of seeds per plant. These results also suggest that elicitors could be used not only to increase isoflavone concentration of soybean seeds but also seed yield [16]. Saini et al., reported that salicylic acid is a phenolic compound that plays a role in regulating plant growth khsusnya physiological activity such as photosynthesis, metabolism of nitrate, ethylene production, flowering and protects from both biotic and abiotic stress.

TABLE 1: Seed dry weight of soybean cultivars with different type and application time of elicitor.

Cultivar	Elicitor	Time of application		Mean
		V4 (T1)	R3 (T2)	
Anjasmoro (V1)	No elicitor (E0)	9.28	9.37	9.33
	Chitosan (E1)	7.35	9.99	8.67
	Methyl jasmonate (E2)	6.12	9.67	7.90
	Salicylic acid (E3)	11.87	10.27	11.07
Mean V1 x T		8.66c	9.83b	
Mean V1				9.24b
Wilis (V2)	No elicitor (E0)	13.73	9.44	11.58
	Chitosan (E1)	10.55	10.63	10.59
	Methyl jasmonate (E2)	11.45	10.20	10.82
	Salicylic acid (E3)	12.38	9.17	10.77
Mean V2 x T		12.02a	9.86b	
Mean V2				10.94a
Mean T	No elicitor (E0)	11.50	9.41	10.46
	Chitosan (E1)	8.95	10.31	9.63
	Methyl jasmonate (E2)	8.78	9.93	9.36
	Salicylic acid (E3)	12.12	9.72	10.92

Different letters at the same of group treatment represent significant differences at Duncan's Multiple Range Test ( $P < 0.05$ ).

TABLE 2: Number of filled pod of soybean cultivars with different type and application time of elicitor.

Cultivar	Elicitor	Time of application		Mean
		V4 (T1)	R3 (T2)	
Anjasmoro (V1)	No elicitor (E0)	44.13	45.92	45.02
	Chitosan (E1)	32.00	45.92	38.96
	Methyl jasmonate (E2)	24.83	34.50	29.67
	Salicylic acid (E3)	37.75	58.88	48.31
Mean V1 x T		34.68d	46.30c	
Mean V1				40.49b
Wilis (V2)	No elicitor (E0)	62.08	55.58	58.83
	Chitosan (E1)	66.00	45.83	55.92
	Methyl jasmonate (E2)	73.63	48.58	61.10
	Salicylic acid (E3)	67.50	62.13	64.81
Mean V2 x T		67.30a	53.03b	
Mean V2				60.17b
Mean T	No elicitor (E0)	53.10	50.75	51.93
	Chitosan (E1)	49.00	45.88	47.44
	Methyl jasmonate (E2)	49.23	41.54	45.39
	Salicylic acid (E3)	52.63	60.50	56.56

Different letters at the same group treatment represent significant differences at Duncan's Multiple Range Test ( $P < 0.05$ ).

TABLE 3: The dry weight of 100 seeds of soybean cultivars with different type and application time of elicitor.

Cultivar	Elicitor	Time of application		Mean
		V4 (T1)	R3 (T2)	
Anjasmoro (V1)	No elicitor (E0)	11.76	12.59	12.17
	Chitosan (E1)	11.50	12.50	12.00
	Methyl jasmonate (E2)	12.00	11.38	11.69
	Salicylic acid (E3)	10.72	12.35	11.53
Mean V1 x T		11.50a	12.20a	
Mean V1				11.85a
Wilis (V2)	No elicitor (E0)	9.80	9.18	9.49
	Chitosan (E1)	9.25	8.90	9.08
	Methyl jasmonate (E2)	10.13	9.32	9.72
	Salicylic acid (E3)	10.02	8.20	9.11
Mean V2 x T		9.80b	8.90b	
Mean V2				9.35b
Mean T	No elicitor (E0)	10.78	10.88	10.83
	Chitosan (E1)	10.38	10.70	10.54
	Methyl jasmonate (E2)	11.07	10.35	10.71
	Salicylic acid (E3)	10.37	10.27	10.32

Different letters at the same group treatment represent significant differences at Duncan's Multiple Range Test ( $P < 0.05$ ).

### 3.2. Number of the filled pod

Mean comparisons for a number of a filled pod of soybean cultivars with different type and time of application of elicitor are shown in Table 2. Cultivar Wilis is a significantly higher number of filled pod per plant (60.17 pod) than Anjasmoro (40.49 pod). In Anjasmoro cultivar, application of elicitor on V4 and R3 produced a lower number of filled pod per plant than the Wilis cultivar on V4 and R3 elicitor treatment (Table 2).

Salicylic acid treatments tend to produce the highest number of the filled pod. In Anjasmoro cultivar, the elicitor treatment of salicylic acid at R3 tend to produce the highest number of the filled pod (58.88 pod), while in Wilis cultivar, the elicitor treatment of methyl jasmonate at V4 tend to produce the highest number of the filled pod (73.63 pod). It suggests that the response of each cultivar to the treatment type and timing of elicitor application is different.

### 3.3. Dry weight of 100 seeds

Mean comparisons for dry weight of 100 seeds of soybean cultivars with different type and time of application of elicitor are shown in Table 3. Cultivar Anjasmoro is the significantly higher dry weight of 100 seeds than Anjasmoro (11.85 g) than Wilis (9.35 g). In Anjasmoro cultivar, application of elicitor on V4 and R3 tend to produce a higher dry weight of 100 seeds than the Wilis cultivar on V4 and R3 elicitor treatment (Table 3).

The yield response was observed with the application of chitosan at the R3 stage, which resulted in yield increases in Anjasmoro cultivar. Otherwise, previous research by Al Tawaha, et al. [16] reported that the most consistent yield response was observed with the application of chitosan at the V4 stage, which resulted in yield increases in three of four cultivar x year combinations. Other studies, mainly with chitosan, yield increases in a range of plants following elicitor applications. For example, Hirano et al. [17] reported a 20 % seed yield increase in field-grown soybean following seed treatment with chitosan. Pieta and Pastucha [18] also reported yield increases in soybean following foliar application of chitosan at the initial stages of flowering.

The most frequently reported response of plants, following treatment with elicitors, is increased pest resistance, which is mainly attributable to an increased accumulation of phenolic compounds and pathogenesis-related proteins [11,19–21].

## 4. Conclusion

Base on the research, it concluded that the response of each cultivar to the treatment type and timing of elicitor application is different. In Anjasmoro cultivar, the elicitor treatment of salicylic acid at R3 tend to produce the highest number of the filled pod (58.88 pod), while in Wilis cultivar, the elicitor treatment of methyl jasmonate at V4 tend to produce the highest number of the filled pod (73.63 pod). The yield response of dry weight of 100 seeds was observed with the application of chitosan at the R3 stage.

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