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Physiological Role of Benzoic Acid and Salicylic Acid on Growth, Yield, Some Biochemical and Antioxidant Aspects of Soybean Plant

Mervat Sh. Sadak, Safaa R. El-Lethy and Mona G. Dawood

Botany Department, National Research Centre, Dokki, Giza, Egypt, P.O. Box: 12622

Abstract: Benzoic acid is a biosynthetic precursor of salicylic acid. Salicylic acid is an endogenous growth regulator of phenolic nature, which participates in the regulation of physiological processes in plants. Two pot experiments were conducted at the greenhouse of the National Research Centre, Dokki, Giza, Egypt during two successive summer seasons (2011 and 2012) to study the effect of exogenous application of benzoic acid and salicylic acid at different concentrations (100, 200 and 400 mg/l) on vegetative growth characters, photosynthetic pigments, seed yield and yield components as well as seed biochemical constituents and fatty acids composition of the yielded oils. Exogenous application of benzoic acid and salicylic acid led to marked increases in growth characters (plant height, branches and leaves number/plant as well as plant dry weight), photosynthetic pigments (chlorophyll a, chlorophyll b, chlorophyll (a+b), carotenoids and consequently total pigments). All treatments increased seed yield and its components (number and dry weight of pods/plant, seeds number/pod, seeds weight/plant and seed index) as well as seed biochemical constituents (protein, carbohydrate, oil, flavonoid and phenolic content). Different treatments improved oil quality as it affected fatty acids composition of soybean oil. Salicylic acid and benzoic acid caused increases in total unsaturated fatty acids and total essential fatty acids accompanied by decreases in total saturated fatty acids. It is worthy to mention that promotive effect of benzoic acid was more pronounced than salicylic acid in increasing most of the tested parameters of soybean plant. Moreover, benzoic acid at 400 mg/l was the most effective treatment.

Key words: Benzoic Acid · Fatty Acids · Flavonoid · *Glycine max* · Nutritive Value · Oil · Salicylic Acid

INTRODUCTION

Soybean [*Glycine max* (L.) Merril] is an important widely used oil seed and protein crop of the world. It is referred to "the miracle golden bean" because of its high nutritive value containing about 35% protein, 20% edible oil and 35% carbohydrates [1]. Soybean oil is one of the common vegetable oils containing a significant amount of unsaturated fatty acids: α-linolenic acid (omega-3 acid); linoleic acid (omega-6 acid) and oleic acid (omega-9 acid). Its oil is also used as raw material in manufacture of antibiotics, paints, varnishes, adhesives, lubricants *etc.* [2]. A proportion of the crop is consumed directly as human food. However, soybean products appear in large variety of processed foods [3]. Some soybean varieties can be profitably used as green manure and fodder. Soybean also helps in improving soil fertility and productivity by fixing atmospheric nitrogen through *Rhizobium* bacteria that live in root nodules. Increasing plant productivity is one of the main targets in Egyptian agricultural policy; this could be achieved through fertilization and/or antioxidant and plant growth regulators treatments. Plant growth regulators can improve the physiological efficiency including photosynthetic ability and enhance source-sink relationship thus stimulate translocation of photo-assimilates and helping in effective flower formation, fruit and seed development and ultimately enhance productivity of the crops [4]. Salicylic acid is an endogenous growth regulator and acts as non-enzymatic antioxidant of phenolic nature, which participates in the regulation of physiological processes in plants. Salicylic acid or ortho-hydroxybenzoic acid is distributed in a wide range of plant species. The biosynthesis of salicylic acid in plants starts from

Corresponding Author: Mervat Sh. Sadak, Botany Department, National Research Centre, Dokki, Giza, Egypt, P.O. Box: 12622.

phenylalanine and follows one of two known paths of synthesis which involves trans-cinnamic acid then hydroxylation of benzoic acid which is a direct precursor of salicylic acid [5]. Salicylic acid has direct effects on plant growth, flower induction and uptake of ions. It affects on ethylene biosynthesis, stomata movement and also reverses the effects of abscisic acid on leaf abscission. In addition, enhancement the level of chlorophyll and carotenoids pigments, photosynthetic rate and modifying the activity of some of the important enzymes are assigned to salicylic acid [6, 7]. Benzoic acid is a biosynthetic precursor of salicylic acid and has been tested in different crops [5]. Benzoic acid is potentially known to provide abiotic stress tolerance [8]. However, the effect of benzoic acid on growth and yield of plant has not been widely studied until now. Therefore, the present study was undertaken to investigate the possible role of benzoic acid and salicylic acid in improving growth, development, some physiological attributes, yield and nutritional values of the yielded soybean seeds.

MATERIALS AND METHODS

Experimental Procedure: Two pot experiments were carried out at the greenhouse of the National Research Centre, Dokki, Giza, Egypt during two successive summer seasons (2011 and 2012) to study the effect of exogenous application of benzoic acid and salicylic acid separately on growth, yield and some biochemical constituents of soybean (*Glycine max* L. cv. Giza 111). Soybean seeds were obtained from Oilseed Department, Agricultural Research Centre, Giza, Egypt. Soybean seeds were selected for uniformity by choosing those of equal size and with the same color. The selected seeds were washed with distilled water, sterilized with 1% sodium hypochlorite solution for about 2 minutes and thoroughly washed again with distilled water. Five uniform air dried soybean seeds were sown along a centre row in each pot at 30-mm depth in 40 cm diameter pots, each pot filled with about 7 kg of mixture of clay and sandy soils at the ratio of 3:1 (w/w). At sowing, a granular commercial rhizobia was incorporated into the top 30-mm of the soil in each pot with the seeds. Nitrogen, phosphorous and potassium fertilizers were added at the recommended doses by mixing thoroughly into the soil of each pot immediately before sowing. At 30 days after sowing (DAS), the seedlings were thinned to leave two seedlings per pot. The pots were arranged in a complete randomized design with ten replicates for each treatment. The plants were sprayed twice with benzoic acid and salicylic acid at different concentrations (100, 200 and 400 mg/l), while control plants were sprayed with distilled water during vegetative growth at 45 and 60 days after sowing.

Data Recorded: Two weeks after the second spraying (at the beginning of flowering stage) plant samples were collected to determine plant height; number of branches and leaves / plant and dry weight /plant as well as photosynthetic pigments. At harvest, the following items were estimated: number of pods/ plant, weight of pods/ plant, number of seeds/ pod, weight of seeds/plant and seed index. Air dried seeds were ground into fine powder and kept in desiccators for analysis.

Chemical Analysis: Photosynthetic pigments (chlorophyll a, chlorophyll b, chlorophyll (a+b) and carotenoids) in fresh leaves were determined according to Moran [9]. Seed oil contents were determined using Soxhlet apparatus and petroleum ether (40-60°C) as a solvent according to AOAC [10]. The resultant defatted meal is used for determination of proteins, carbohydrates, phenolic compounds, tannins and flavonoids. Protein contents were determined by micro-kjeldahl method according to Miller and Houghton [11]. Total carbohydrates were determined colorimetrically according to the method of Herbert *et al*. [12]. Phenolic compounds were determined by using Folin and Ciocalteau phenol reagent as the method described by Makkar *et al*. [13]. Tannins were determined using the modified vanillin hydrochloric acid (MV-HCl) as reported by Maxson and Rooney [14]. Total flavonoid contents were measured by the aluminum chloride colorimetric assay as described by Ordoñez *et al*. [15]. Methyl esters of fatty acids were prepared from an aliquot of total lipid according to Harborne [16]. Identification and quantitative determination of fatty acids were performed using Gas Liquid Chromatography.

Statistical Analysis: Data were statistically analyzed using the least significant difference at 5% level of probability according to Snedecor and Cochran [17].

RESULTS

Plant Growth Characters: Results presented in Table 1 clearly reveal that, using benzoic acid and salicylic acid as foliar treatment at different concentrations (100, 200 and 400 mg/l) increased gradually all studied growth characters (plant height, number of branches and leaves/plant as well as plant dry weight) of soybean plant as compared with control plant. These increases were

Table 1: Effect of benzoic acid and salicylic acid on growth characters of soybean plant at 75 days after sowing

Treatments		Plant height (cm)	Branches number/plant	Leaves number/plant	Plant dry weight (g)
Control		52.33^{d}	7.00^{b}	10.00^{c}	4.91^{e}
Benzoic acid (mg/l)	100	73.33^{b}	7.67^{ab}	12.00^{b}	5.50^{de}
	200	78.00^{b}	7.67^{ab}	13.00^{ab}	6.15^{cde}
	400	85.33^{a}	8.77^{a}	14.66^{a}	7.69^{ab}
Salicylic acid (mg/l)	100	61.67^{c}	7.67^{ab}	11.67^{bc}	6.57^{bcd}
	200	64.67^{c}	8.33^{ab}	14.00^{a}	6.92^{abc}
	400	65.67^{c}	9.00^{a}	14.33^{a}	8.21^{a}

Data are means of two seasons -Means followed by the same letter for each tested parameter are not significantly different by Duncan's test ($P < 0.05$)

Table 2: Effect of benzoic acid and salicylic acid on photosynthetic pigments (mg/g fresh weight) of soybean plant at 75 days after sowing

Treatments		Chlorophyll a	Chlorophyll b	Total chlorophylls	Carotenoids	Total pigments
Control		1.29^{c}	0.83^{e}	2.12^{f}	0.21^{e}	2.33^{f}
Benzoic acid (mg/l)	100	1.67^{b}	1.28^{c}	2.95^{c}	0.56^{c}	3.51^{c}
	200	2.05^{a}	1.29^{c}	3.34^{b}	0.75^{b}	4.09^{b}
	400	2.09^{a}	1.45^{b}	3.54^{a}	0.96^{a}	4.50^{a}
Salicylic acid (mg/l)	100	1.32^{c}	1.07^{d}	2.39^{e}	0.23^{e}	2.62^{e}
	200	1.40^{c}	1.39^{b}	2.79^{d}	0.25^{e}	3.04^{d}
	400	1.65^{b}	1.60^{a}	3.25^{b}	0.29^{d}	3.54^{c}

Data are means of two seasons -Means followed by the same letter for each tested parameter are not significantly different by Duncan's test ($P < 0.05$)

significant in plant dry weight at all concentrations, except at 100 and 200 mg/l benzoic acid. Application of 400 mg/l benzoic acid gave the highest plant height and leaves number/plant. They were increased by 63.06% and 46.60%, respectively, relative to control plant. While, the highest branches number/plant and plant dry weight were recorded at 400 mg/l salicylic acid. They were increased by 28.57% and 67.21%, respectively as compared to control plant.

Photosynthetic Pigments: The effect of benzoic acid and salicylic acid at different concentrations (100, 200 and 400 mg/l) on photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll (a+b), carotenoids and total pigments) of soybean plant are shown in Table 2. Data show that different concentrations of benzoic acid and salicylic acid significantly increased chlorophyll a, chlorophyll b, total chlorophyll (a+b), carotenoids and consequently total pigments, except the increases in chlorophyll a and carotenoids contents at 100 and 200 mg/l salicylic acid were not significant. Table 2 clearly shows that the effect of benzoic acid was more pronounced than salicylic acid. In addition, 400 mg/l of benzoic acid was the most effective treatment, since it increased chlorophyll a by 62.01%, chlorophyll (a+b) by 66.98% and total pigments by 93.13%. In the meantime, 400 mg/l salicylic acid was the most effective treatment in increasing chlorophyll b by 92.77% over control plant.

Yield and Yield Attributes: Data in Table 3 show that, seed yield and its components such as number and dry weight of pods/plant, seeds number/pod, seeds weight/plant and seed index were increased by foliar application of benzoic acid and salicylic acid as compared with control plants. Increasing concentrations of benzoic acid and salicylic acid caused gradual increases in all the above mentioned parameters. In addition, 400 mg/l of benzoic acid was the most effective treatment. It increased pods number/plant by 65.19%, pods dry weight/plant by 89.23%, seeds number/pod by 11.37% and seeds weight/plant by 72.13%, meanwhile, 400 mg/l salicylic acid was the most effective treatment in increasing seed index by 18.02% as compared with control plant.

Nutritive Value of the Yielded Seeds: Data in Table 4 indicate that foliar application of benzoic acid and salicylic acid led to significant increases in the biochemical constituents of the yielded soybean seeds as compared with control plant. Moreover, the values of protein, carbohydrate and oil were gradually increased with increasing concentrations of benzoic acid and salicylic acid. The maximum increases in protein, carbohydrate and oil contents of the yielded soybean seeds were obtained by 400 mg/l benzoic acid as it reached to 11.92% for protein, 17.61% for carbohydrate and 40.23% for oil relative to control plant.

Table 3: Effect of benzoic acid and salicylic acid on seed yield and yield components of soybean plant

Treatments		Pods number /plant	Pods dry weight/plant (g)	Seeds number/pod	Seeds weight/ plant	Seed index
Control		12.21^{e}	4.83^{c}	1.67^{b}	3.66^{c}	13.54^{c}
Benzoic acid (mg/l)	100	16.70^{bc}	6.80^{b}	1.69^{ab}	5.38^{b}	14.91^{ab}
	200	18.17^{ab}	7.80^{b}	1.73^{ab}	5.62^{ab}	15.19^{ab}
	400	20.17^{a}	9.14^{a}	1.86^{a}	6.30^{a}	15.66^{ab}
Salicylic acid (mg/l)	100	13.25^{de}	5.44^{c}	1.62^{b}	4.89^{b}	14.64^{bc}
	200	15.83^{bcd}	7.60^{b}	1.75^{ab}	4.99^{b}	15.15^{ab}
	400	15.85^{bcd}	7.68^{b}	1.67^{b}	4.79^{b}	15.98^{a}

Data are means of two seasons -Means followed by the same letter for each tested parameter are not significantly different by Duncan's test ($P < 0.05$)

Table 4: Effect of benzoic acid and salicylic acid on nutritive value and antioxidant substances of the yielded soybean seeds

Treatments		Protein %	Carbohydrate %	Oil %	Flavonoid mg/g	Phenolic contents %	Tannins mg/100g
Control		36.90^{e}	27.36^{d}	18.22^{d}	5.24^{c}	1.26^{c}	18.14^{a}
Benzoic acid (mg/l)	100	38.87^{cd}	30.86^{bc}	23.64^{b}	6.28^{bc}	1.52^{ab}	16.89^{d}
	200	40.38^{abc}	31.37^{ab}	24.30^{ab}	6.80^{ab}	1.54^{a}	16.72^{d}
	400	41.30^{a}	32.18^{a}	25.55^{a}	8.30^{a}	1.55^{a}	15.14^{f}
Salicylic acid (mg/l)	100	38.12^{de}	30.12^{c}	21.46^{c}	6.25^{bc}	1.42^{b}	17.64^{b}
	200	39.56^{bcd}	31.54^{ab}	23.54^{b}	6.30^{bc}	1.46^{ab}	17.16^{c}
	400	41.11^{ab}	31.53^{ab}	24.55^{ab}	7.25^{ab}	1.55^{a}	16.49^{e}

Data are means of two seasons -Means followed by the same letter for each tested parameter are not significantly different by Duncan's test ($P < 0.05$).

Table 5: Effect of benzoic acid and salicylic acid on fatty acids composition of the yielded soybean oil

	Treatments						
		Benzoic acid (mg/l)			Salicylic acid (mg/l)		
Fatty acids %	Control	100	200	400	100	200	400
C16:0	14.74	13.83	11.80	10.13	12.53	11.91	11.38
C18:0	4.85	4.97	4.92	4.44	4.19	5.37	5.97
C18:1	20.51	17.88	20.73	21.43	21.29	20.50	20.89
C18:2	48.38	51.31	52.28	54.78	50.19	49.89	51.08
C18:3	7.89	8.43	6.98	5.46	7.31	8.02	5.93
C20:0	0.59	0.54	0.56	0.64	1.19	1.53	1.21
C22:0	1.33	1.32	1.23	0.93	1.59	1.28	1.75
C24:0	0.26	0.22	--	0.29	0.19	--	0.37
TEFA	56.27	59.74	59.26	60.24	57.50	57.91	57.01
TS	21.77	20.88	18.51	16.43	19.69	20.09	20.68
TUS	76.78	77.62	79.99	81.67	78.79	78.41	77.90
TUS/TS	3.53	3.72	4.32	4.97	4.00	3.90	3.77

C16:0 (Palmitic acid), C18:0 (Stearic acid), C18:1(Oleic acid), C18:2 (Linoleic acid), C18:3 (Linolenic acid), C20:0 (Arachidic acid), C22:0 (Behenic acid), C24:0 (Lignoceric acid), TEFA (Total Essential Fatty Acids), TS (Total Saturated), TUS (Total Unsaturated)

Antioxidant Substances of the Yielded Seeds: Data in Table 4 clearly show that, foliar application of benzoic acid and salicylic acid at different concentrations (100, 200 and 400 mg/l) significantly increased flavonoid content and phenolic content except at 100 mg/l benzoic acid and 100 and 200 mg/l salicylic acid, that showed insignificant increases in flavonoid content as compared with control plant. Benzoic acid at 400 mg/l was the most effective treatment in increasing the two tested parameters and the percentage of increase in flavonoid contents was 58.39% and in phenolic contents was 23.01% as compared to control plant. On the other hand, the different treatments significantly decreased tannins content especially at 400 mg/l benzoic acid that gave the highest decrease in tannins content by 16.54% as compared with control plant. It is clear that benzoic acid was more effective than salicylic acid in increasing values of flavonoid and phenolic contents as well as decreasing tannins values.

Fatty Acids Composition of the Yielded Oil: Data presented in Table 5 show the main fatty acids composition of the yielded soybean oil. These fatty acids are Palmitic (C16:0), Stearic (C18:0), Oleic (C18:1), Linoleic (C18:2), Linolenic (C18:3), Arachidic (C20:0), Behenic

(C22:0) and Lignoceric (C24:0). The predominant saturated fatty acid was Palmitic acid and the predominant unsaturated fatty acid was Linoleic acid followed by Oleic acid. Benzoic acid and salicylic acid at different concentrations (100, 200 and 400 mg/l) induced marked increases in the levels of total unsaturated fatty acids accompanied by decrease in total saturated fatty acids. The most effective treatment was 400 mg/l benzoic acid, as it caused the highest increases in total unsaturated fatty acids, total essential fatty acid (Linoleic acid +Linolenic acid) and ratio of total unsaturated/total saturated (TUS/TS) as well as the highest decrease in total saturated fatty acids.

DISCUSSION

Improving productivity of crop plants by exogenous application of some potential growth regulators is considered an effective technique. Salicylic acid and its precursor benzoic acid have been reported to induce significant effects on various biological aspects in plants. These compounds influence in a variable manner, inhibiting certain processes and enhancing others [5]. Our results exhibited that benzoic acid and salicylic acid have stimulatory effects on vegetative growth parameters of soybean plant (Table 1). The obtained results of benzoic acid are in agreement with those reported by Tuna *et al.* [18] on maize and Anjum *et al*. [19] on soybean. Regarding salicylic acid, our results are similar to those reported by Salarizdah *et al*. [20] on canola, Dawood *et al.* [21] on sunflower and Ali and Mahmoud [22] on mung bean. The stimulatory effect of benzoic acid and salicylic acid on soybean growth could be attributed to their stimulatory effect on photosynthesizing tissue [23]. The increases in dry weights of soybean plant might be attributed to the increase in number of branches and leaves leading to increase in photosynthetic process. Moreover, the promotive effect of salicylic acid and its precursor benzoic acid could be attributed to their bioregulator effects on physiological and biochemical processes in plants such as ion uptake, cell elongation, cell division, cell differentiation, sink/source regulation, enzymatic activities, protein synthesis and photosynthetic activity as well as increase the antioxidant capacity of plants [24]. Chlorophyll pigments play a key role in light capturing for photosynthesis, whose content forced a direct impact on the intensity of photosynthesis. The stimulative effect of benzoic acid at different concentrations on photosynthetic pigments as shown in Table 2 are similar to those of Anjum *et al.* [19] on soybean and attributed the improvement effect of benzoic acid to increase gas exchange attributes, stomatal conductance, transpiration rate and photosynthetic rate. The stimulatory effects of salicylic acid on photosynthetic pigments of soybean are in agreement with those of Barakat [25] on wheat and Saeidnejad *et al.* [26] on maize. Fariduddin *et al.* [27] stated that salicylic acid enhanced the net photosynthetic rate, intercellular CO_2, water use efficiency, stomatal conductance and transpiration rate in *Brassica juncea*. In addition, the enhancing effects of salicylic acid on photosynthetic capacity could be attributed to its stimulatory effects on Rubisco activity and pigment contents as well as increased CO_2 assimilation, photosynthetic rate and increased mineral uptake by the plant [28]. Moreover, its antioxidant scavenging effect protected chloroplasts and prevented chlorophyll degradation by the toxic reactive oxygen radicals [29]. Regarding soybean yield and its attributes, Table 3 shows that benzoic acid and salicylic acid increased seed yield and yield components as compared with control plants. These results are in good agreement with those obtained by Bukhsh *et al.* [30] on sunflower, Abdel-Hakim *et al.* [31] on snap bean and Anjum *et al*. [19] on soybean. The increase in the seed yield could be a reflection of the effect of bioregulators on growth and development, it might be due to (a) marked increase in the number of branches per plant (Table 1) which gave a chance to the plant to carry more flowers, pods and hence more seeds (b) marked increase in the photosynthetic pigments content (Table 2), which could lead to increase in photosynthesis, resulting in greater transfer of photo-assimilates to the seeds and causing increase in their weight (Table 3). Table 4 reveals that different concentrations of benzoic acid and salicylic acid increased significantly and gradually the biochemical contents (protein, carbohydrate and oil) of the yielded soybean seeds. These results are in agreement with those obtained by Al-Hakimi [32] on soybean, Noreen and Ashraf [33] on sunflower. Nandini *et al.* [34] on soybean, Bakry *et al.* [35] on linseed and Sadak and Abd Elhamid [36] on flax. This stimulatory effect of benzoic acid and salicylic acid might be attributed to their effects on enzymatic activity and translocation of the metabolites to soybean seed. The substantial increase in carbohydrate contents may be due to the activation of photosynthetic machinery, as a result of the stimulatory effects of the used plant growth biostimulators on photosynthetic process. The increment in oil percentage may be due to the increase in vegetative growth and nutrients uptake [30].

Antioxidant compounds (flavonoid and phenolic contents) of soybean were increased in response to different concentrations of benzoic acid and salicylic acid (Table 4). These increments in flavonoid and phenolic contents may be due to the phenolic nature of benzoic acid and salicylic acid [5]. Therefore, it could be expected that their application on plants increased flavonoid and phenolic concentration as previously mentioned by Zaghlool *et al.* [37] in bean and Bakry *et al.* [35] on linseed. The increase in flavonoid and total phenolic contents in response to all treatments may be due to the increase in carbohydrate synthesis [38]. Moreover, Dawood and Sadak [39] showed that the increase in total phenolic contents was concur with the increase in IAA contents and led to the suggestion that most of phenolic compounds are diphenols and polyphenols which may inhibit IAA oxidase activity and leading to auxin accumulation and reflected in stimulating the growth and yield of plant. With regard to the effect of benzoic acid and salicylic acid on fatty acids composition of the yielded oil (Table 5). The obtained results are quite similar to those obtained by Noreen and Ashraf [33], Bakry *et al*. [35] and Sadak and Abd Elhamid [36]. Abdel Rahim *et al*. [40] reported that the increase in percentage of unsaturated fatty acids accompanied by the decrease in saturated fatty acids proved the quality of oil. Polyunsaturated fatty acids from soybean oil are essential for human diet because of lowering the risk of heart diseases related to cholesterol oxidation. In addition, consumption of Oleic, Linoleic and Linolenic acids lowers the level of low density lipoprotein (LDL) in human blood.

CONCLUSION

Foliar application of different concentrations of benzoic acid and salicylic acid on soybean plant increased growth, photosynthetic pigments and seed yield quantity and quality. The effect of benzoic acid was more pronounced than salicylic acid in increasing most studied parameters. Moreover, 400 mg/l of benzoic acid was the most effective treatment.

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