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Effect of Salicylic Acid and Benzoic Acid on Growth, Yield and Some Biochemical Aspects of Quinoa Plant Grown in Sandy Soil

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Abstract: A field experiment was carried out at the Agricultural Production and Research Station, National Research Centre (NRC), Nubaria Province, Egypt during two successive winter seasons. In order to study the effects of exogenous application of benzoic acid and salicylic acid at different concentrations (200, 200 and 400 mg/l) on vegetative growth characters, some biochemical aspects, seed yield quantity and quality and seed biochemical constituents of quinoa plant. In general, exogenous application of benzoic acid and salicylic acid led to marked increases in growth characters (plant height, leaves number/plant as well as plant dry weight) concomitantly with an increase in the levels of IAA, photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids, phenol, free amino acid contents, total soluble sugar, total carbohydrates and yield components. All treatments increased seed yield and the nutritional values of the yielded seed (carbohydrate contents, protein, oil, flavonoids, phenolic content and antioxidant substances). 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 quinoa plant. Moreover, benzoic acid at 400 mg/l was the most effective treatment.
Key words: - Benzoic acid- Biochemical aspects - Quinoa - Salicylic acid

Introduction

Quinoa (*Chenopodium quinoa*), another pseudocereal from the Andean origin, is extensively cultivated at mountain altitudes in Peru and Bolivia. Based on both their higher protein content than conventional used cereal grains and their higher protein nutritional quality[1], they have been gained an increased interest in recent years. On the basis of their nutritional values (rich in protein, fat, dietary fiber, ash, and minerals, especially calcium and sodium and contain a higher amount of lysine than conventional cereals), quinoa has been used as important ingredients primarily in bread, pasta, and baby's food[2]**.** Quinoa is a species that can tolerate different stresses such as salinity, cold air, high solar radiation, night subfreezing temperatures, and different soil pHs[3]**.** It can also grow in arid and semiarid regions, lowlands, brackish lands, and salt-water marshes[4]. However, Gallardo M. et al[5] have demonstrated that soil moisture plays an important role in determining the time and rate of quinoa seed germination and seedling growth.

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[6]. Benzoic acid is naturally synthesized by plants and classified in the group of carboxylic acids. These organic compounds are exudated toward the rhizosphere to facilitate the assimilation of mineral nutrients, also associated with the elevation of soil weathering and mineral lixiviation rate[7]. The most common carboxylic acids in the soil include oxalate, acetate, citrate, and formate[8] although benzoic acid is also present in minor quantities[7]. In addition, benzoic acid seems to work like a stress signaling compound, when it is applied in a low concentration range (less than one millimolar) it induces tolerance to saline stress in cabbage and tomato plants[9]. Benzoic acid also considered as a biosynthetic precursor of salicylic acid and has been tested in different crops[10]. Benzoic acid is potentially known to provide abiotic stress tolerance[11]. However, the effect of benzoic acid on growth and yield of plant has not been widely studied until now. 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 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[10]. 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[12,13].

The experiments will be carried at sandy soils in Egypt. This region, as a part of the Sahara Desert of Northern Africa, is exposed to a combination of environmental stress conditions including low water availability, high irradiances, temperature fluctuations, soil salinity and nutrients deprivation. Therefore, the present study investigate the ability of quinoa plant's grown sandy soil conditions and to study the possible role of benzoic acid and salicylic acid in improving growth, development, some physiological attributes, yield and nutritional values of the yielded of quinoa seeds.

Materials and Methods

Plant material and growth conditions:

A field experiment was conducted at the Experimental Station of National Research Centre, Nubaria district Beheira Governorate, Egypt, during two successive seasons. The soils of both experimental sites were reclaimed sandy soil where mechanical and chemical analysis is reported in Table (1) according to[14]**.**

Table 1: Mechanical and chemical analysis of the experimental soil sites.

A. Mechanical analysis:

Sand		**Silt 20-0µ%**	**Clay < 2µ%**	**Soil texture**
Course 2000-200µ%	Fine 200-20µ %			
47.46	36.19	12.86	4.28	Sandy

B. Chemical analysis:

pH 1:2.5	**EC dSm^{-1}**	**$CaCO_3$**	**OM %**	**Soluble Cations meq/l**				**Soluble anions meq/l**			
				Na^+	**K^+**	**Mg^+**	**Ca^{++}**	**CO_3^{--}**	**HCO_3^-**	**Cl^-**	**SO_4^{--}**
7.60	0.13	5.3	0.06	0.57	0.13	0.92	1.0	0.0	1.25	0.48	0.89

Available nutrients						
Macro element ppm			Micro element ppm			
N	P	K	Zn	Fe	Mn	Cu
52	12.0	75	0.14	1.4	0.3	0.00

Seeds of quinoa (*Chenopodium quinoa* Willd.) were obtained from Agricultural Research Centre Giza, Egypt. The experimental design was in randomized complete block with four replications, quinoa seeds were sown on November in both seasons at the rate of 60 kg/faddan in rows 3.5 meters long, and the distance between rows was 20 cm apart. Plot area was 10.5 m^2 (3.0 m in width and 3.5 m in length). The recommended agricultural practices of growing quinoa were applied. Pre-sowing, 150 kg/feddan of calcium super-phosphate (15.5% P_2O_5) was applied to the soil. Nitrogen was applied after emergence in the form of ammonium nitrate 33.5% at a rate of 75 Kg/feddan in five equal doses before the 1st, 2nd, 3rd, 4th and 5thirrigation. Potassium sulfate (48.52 % K_2O) was added in two equal doses of 50 kg/feddan, before the 1^{st} and 3^{rd}irrigations. Irrigation was carried out using the new sprinkler irrigation system where water was added every 7 days.

The plants were sprayed twice with benzoic acid and salicylic acid at different concentrations (200, 300 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 75 days from sowing plant samples were collected to determine plant height; number of leaves / plant, fresh and dry weight of shoot and root /plant as well as some biochemical aspects in leaves photosynthetic pigments, indole acetic acid contents total phenol contents, total free amino acid, carbohydrate constituents (total soluble sugars, polysaccharides, total carbohydrates).

At harvest, the following items were estimated: shoot length, fruiting branch numbers, shoot weight, seeds weight/plant and 1000 seed weight. Air dried seeds were ground into fine powder and kept in desiccators for analysis. Some chemical parameters are measured in the yielded grains as proteins %, carbohydrates %, flavonoids, antioxidant activity, total phenol and oil %.

Chemical analysis:

Photosynthetic pigments: Total chlorophyll a and b and carotenoids contents in fresh leaves were estimated using the method of Lichtenthaler H.K. et al[15]. Indole acetic acid content were extracted and analyzed by the method of Larsen, P.A. et al[16]. Total phenol content, the extract was extracted as IAA extraction, and then measured as described by Danil, A.D.et al[17]. Free amino acid was determined with the ninhydrin reagent method Yemm & Cocking [18].Total soluble sugars (TSS), was analyzed by Yemm and Willis[19]. Determination of total carbohydrates was carried out according to Herbert D et al[20]. Total protein concentration of the supernatant was determined according to the method described by Badford M.M.[21]. The oil was extracted according to Kates M et al[22]. Total flavonoids were determined using the method reported by Chang C.et al[23]. The antioxidant activity (DPPH radical scavenging) was determined using the method of Liyana-Pathiranan CM. et al[24].

Statistical analysis:

The data were statistically analyzed on complete randomized design system according to[25]. Combined analysis of the two growing seasons was carried out. Means were compared by using least significant difference (LSD) at 5% levels of probability.

Results and Discussion

Growth parameters:

Results presented in Table 1 reveal that, using benzoic acid and salicylic acid as foliar treatment at different concentrations (200, 300 and 400 mg/l) increased shoot fresh and dry weight as well as root fresh and dry weight of quinoa plant as compared with control plant. While, the highest plant fresh and dry weight (shoot, root) were recorded at 400 mg/l benzoic acid. Application of all treatment caused non significant change in shoot length and number of leaves/plant except shoot length at 300& 400 mg/l benzoic acid and 400 mg/l salicylic acid caused significant increase as compared with control plant respectively.

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[10]. Our results exhibited that benzoic acid and salicylic acid have stimulatory effects on vegetative growth parameters of quinoa plant (Table 1). The obtained results of benzoic acid are in agreement with those reported by[26] on maize and [27]on soybean. Regarding salicylic acid, our results are similar to those reported by Bakry A.B. et al[28] on linseed and [29]on mung bean. The stimulatory effect of benzoic acid and salicylic acid on quinoa plant growth could be attributed to their stimulatory effect on photosynthesizing tissue[30]. The increases in dry weights of quinoa plant might be attributed to the increase in number leaves leading to increase in photosynthetic process Table 2 and endogenous IAA Table 3. Moreover, [31]Blokhina O et al illustrated that, the stimulatory 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.

Table 1: Effect of benzoic acid and salicylic acid on growth parameters of quinoa plant at 75 days after sowing grown in sandy soil.

Material mg/l		Shoot length (cm)	Leaves No /plant	Shoot fresh weight (gm)	Shoot dry weight (gm)	Root fresh weight (gm)	Root dry weight (gm)
Control		17.50	16.33	27.75	3.35	1.87	0.60
Benzoic acid (mg/l)	**200**	18.67	16.33	43.20*	8.5	4.23*	1.10*
	300	20.67*	15.50	47.50*	10.4*	6.03*	1.63*
	400	24.00*	17.33	53.80*	12.8*	7.05*	1.77*
Salicylic acid (mg/l)	**200**	18.3	13.10	32.65*	5.4*	2.90*	0.90
	300	19.33	13.67	34.60*	7.9*	3.03*	1.17*
	400	21.00*	15.50	39.90*	9.3*	5.80*	1.65*
LSD at 5 %		3.02	1.99	2.41	0.98	0.87	0.45

*significant data at 0.05

Photosynthetic Pigments:

The effect of benzoic acid and salicylic acid at different concentrations (200, 300 and 400 mg/l) on photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids and total pigments) of quinoa plant are shown in Table 2. Benzoic acid and salicylic acid significantly increased chlorophyll a chlorophyll b, total carotenoids and consequently total pigments. Increasing benzoic acid and salicylic acid concentrations increased gradually photosynthetic pigments constituents. 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 71.9 %, chlorophyll b by 201%, carrotenoid by 141% and total pigments by 101%. Chlorophyll pigments play a key role in light capturing for photosynthesis, whose content forced a direct impact on the intensity of photosynthesis. The simulative effect of benzoic acid at different concentrations on photosynthetic pigments are similar to those of [27]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 quinoa are in agreement with those of [32]on wheat and [33]on maize. [34]Fariduddin Q. et al stated that salicylic acid enhanced the net photosynthetic rate, intercellular CO, water use efficiency, stomatal conductance and transpiration rate in *Brassica juncea.* In addition, salicylic acid had a stimulatory effect on Rubisco activity and pigment contents as well as increased CO2 assimilation, photosynthetic rate and increased mineral uptake by the plant[35]. Moreover, its antioxidant scavenging effect protected chloroplasts and prevented chlorophyll degradation by the toxic reactive oxygen radicals[36].

Table 2: Effect of benzoic acid and salicylic acid on photosynthetic pigments (µg/g fresh weight) of quinoa plant at 75 days after sowing grown in sandy soil.

Material mg/l		Chlorophyll a	Chlorophyll b	Carotenoids	Total pigments
Control		9.33	1.86	2.01	13.20
Benzoic acid (mg/l)	**200**	11.09*	3.27*	3.23*	17.59*
	300	12.63*	4.09*	3.92*	20.64*
	400	16.04*	5.60*	4.85*	26.48*
Salicylic acid (mg/l)	**200**	10.51	3.20*	2.88*	16.59*
	300	11.43*	3.44*	3.50*	18.37*
	400	14.01*	4.58*	4.79*	23.37*
LSD at 5 %		1.65	1.01	0.84	3.25

*significant data at 0.05

Change in IAA, Phenol and free amino acid contents:

Data in Table 3. Showed that foliar application of benzoic acid and salicylic acid at different concentrations (200, 300 and 400 mg/l) caused significant increases in IAA, total phenol and free amino acid contents .Table 3 clearly shows that the effect of benzoic acid was more pronounced than salicylic acid [37]demonstrated that the osmotic adjustment in plants subjected to stress occurs by the accumulation of high concentrations of osmotically active compounds known as compatible solutes such as proline, glycinebetaine, soluble sugars and free amino acids. They revealed also that such substances play an important role in the adaptation of cells to various adverse environmental conditions through raising osmotic pressure in the cytoplasm, stabilizing proteins and membranes, and maintaining the relatively high water content obligatory for plant growth and cellular functions. Also the increase in amino acid at high drought stressed plants could be due to increased degradation of protein. The increase in IAA content in shoot tissues treated with benzoic acid and salicylic acid concurrent with the increase in growth rate (Table 1) indicates the role of the endogenous hormones in stimulation the cell division and/or cell enlargement and subsequently growth[38]. In a general way, organic acids have been shown to stimulate stress tolerance in plants[39, 40]. Salicylic and benzoic acid are associated with cell wall alterations that increase their hardness, higher synthesis of antioxidants, and elevation of mitochondrial activity[41,42]. Those changes are possibly related with the results observed in the soluble solids of the fruits [43].

Change in carbohydrate contents:

Data in Table 3. show gradual and significant increases in total soluble sugars, polysaccharides and total carbohydrate contents of quinoa plant treated with different concentrations of benzoic acid and salicylic acid (300 and 400 mg/l).While application of benzoic acid or salicylic acid at (200 mg/l) caused non significant changes in all carbohydrates fractions. Application of 400 mg/l benzoic acid or salicylic acid were the most effective treatments as they increased TSS by 39% & 20% , polysaccharides by 91% & 81% and total carbohydrates by 82% & 71% respectively as compared with the untreated plant. Data indicated increases in carbohydrates constituents as affected by different concentrations of benzoic acid and salicylic acid treatments followed the same trend obtained previously on vegetative growth. These increases were gradually increased by increasing concentration of benzoic acid and salicylic acid**.** [44]confirmed that increasing total soluble sugar to reduce osmotic potential is a resistance mechanism of plants against moderate stress. Elevated sugar levels in drought stressed plants may contribute to the turgor maintenance and stabilization of cellular membranes. According to [45]carbohydrates may act as ROS scavengers and contribute to increase in membrane stabilization. The substantial increase in carbohydrate percent 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. SA treatment might also be assumed to inhibit polysaccharide-hydrolyzing enzyme system on one hand and/or accelerate the incorporation of soluble sugars into polysaccharides[28].

Table 3: Effect of benzoic acid and salicylic acid on IAA (µg/g fresh weight), total phenol (mg/100g fresh weight), free amino acid, total soluble sugar, polysaccharides and total carbohydrates (mg/g dry weight) of quinoa plant at 75 days after sowing grown in sandy soil.

Material mg/l		IAA	Phenol	Free amino acid	Total soluble sugar	polysaccharides	Total carbohydrates
Control		13.43	344.7	2.177	26.6	130.2	156.8
Benzoic acid (mg/l)	200	26.02*	423.9*	2.983*	27.4	162.4	189.8
	300	35.24*	465.2*	3.139*	31.5*	187.4*	218.9*
	400	53.75*	542.0*	3.477*	37.0*	248.2*	285.2*
Salicylic acid (mg/l)	200	24.75*	418.9*	2.832*	26.3	156.8	183.1
	300	33.21*	452.1*	3.053*	28.9*	164.2	193.1*
	400	51.89*	511.5*	3.348*	31.9*	236.1*	268.0*
LSD at 5%		1.35	11.55	0.066	1.1	34.9	34.7

*significant data at 0.05

Change in yield and yield components:

Data in Table 4 show that, seed yield and its components such as shoot length, fruiting branches number /plant, shoot weight, seed weight and 1000 seed weigh 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 and salicylic acid were the most effective treatment since it increased shoot length by 68.3% and 61.0 %, fruiting branches number /plant by 73.2% and 48.7, seeds weight/plant by 200% and 146% and 1000 seed weigh by 130% and 110% respectively as compared with control plant. The positive effect of carboxylic acid on yield may be due to the vital effect of this carboxylic acid for stimulating the growth and yield of plant cells. These results are in good agreement with those obtained by[46]on sunflower,[47]on snap bean and [27] 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 fruting branches per plant (Table 4) which gave a chance to the plant to carry more flowers, 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 4). The stimulatory effect were found to be correlated with the increase in content and activity levels of endogenous promoters particularly IAA (table 3) which are known to promote linear growth of plant organs [48].

Table 4: Effect of benzoic acid and salicylic acid on yield and yield components of quinoa plant grown in sandy soil.

Material mg/l		Shoot length (cm)	Fruiting branches No /plant	Shoot weight (g)	Seed weight/ plant (g)	1000 seed weight
Control		49.90	13.67	25.00	4.87	1.02
Benzoic acid (mg/l)	**200**	64.33*	19.33*	55.20*	10.23*	1.32
	300	71.00*	21.67*	62.50*	12.27*	1.78
	400	84.00*	23.67*	63.33*	14.60*	2.35*
Salicylic acid (mg/l)	**200**	59.33*	17.67*	33.43*	8.33*	1.21
	300	69.67*	18.30*	55.00*	11.30*	1.48
	400	80.33*	20.33*	58.50*	12.00*	2.15*
LSD at 5%		2.22	3.43	1.88	1.02	0.90

*significant data at 0.05

Nutritive value of the yielded seeds:

Data in Table 5 indicate that foliar application of benzoic acid and salicylic acid led to significant increases in the biochemical constituents of the yielded quinoa seeds as compared with control plant. Moreover, the values of carbohydrate, protein and oil were gradually increased with increasing concentrations of benzoic acid and salicylic acid. The maximum increases in carbohydrate, protein and oil contents of the yielded quinoa seeds were obtained by 400 mg/l benzoic acid as it reached to 7% for carbohydrate 30% for protein, and 31.6% for oil relative to control plant. These results are in agreement with those obtained by [49] on sunflower.[50] on soybean and [28] on linseed. This stimulatory effect of benzoic acid and salicylic acid might be attributed to their effects on enzymatic activity and translocation of the metabolites to quinoa seed. The substantial increase in carbohydrate percent 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[51]. The increment in oil percentage may be due to the increase in vegetative growth and nutrients uptake[46].

Table 5: Effect of benzoic acid and salicylic acid on nutritive value and antioxidant substances of the yielded seeds of quinoa plant grown in sandy soil.

Material mg/l		Carbohydrates %	Protein %	Oil %	Flavenoids %	DPPH %	Total phenol (mg/100g fr. wt)
Control		60.65	13.18	6.23	61.50	42.95	147.46
Benzoic acid (mg/l)	**200**	61.75	14.52	6.95	65.75*	46.80	172.56
	300	62.89	15.77	7.85*	70.78*	49.95*	205.36*
	400	65.13*	17.24*	8.20*	74.83*	52.85*	241.56*
Salicylic acid (mg/l)	**200**	61.55	13.92	6.65	65.65*	44.98*	170.22
	300	62.17	15.28	7.75*	69.45*	48.98*	189.87*
	400	63.55	16.76*	8.29*	71.74*	51.02*	221.53*
LSD at 5 %		4.21	3.03	0.87	3.74	3.99	36.3

*significant data at 0.05

Antioxidant substances of the yielded seeds

Data in Table 5 clearly show that, foliar application of benzoic acid and salicylic acid at different concentrations (200, 300 400 mg/l) significantly increased flavonoid, phenolic content and antioxidant activity (as DPPH- radical scavenging capacity) as compared with control plant. Benzoic acid at 400 mg/l was the most effective treatment in increasing the tested parameters and the percentage of increase in flavonoid contents was 21.7 %, in phenolic contents was 63.8 % and antioxidant activity was 23% as compared to 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 antioxidant activity values. These increments in flavonoid, phenolic and antioxidant activity contents may be due to the phenolic nature of benzoic acid and salicylic acid[10]. Therefore, it could be expected that their application on plants increased flavonoid, phenolic and antioxidant concentration as previously mentioned by[52] in bean and [28] on linseed. The increase in flavonoid, phenolic and antioxidant in response to all treatments may be due to the increase in carbohydrate synthesis (Table 3) and agreement with[53]**.** Moreover, [54] 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.

Conclusion

Foliar application of different concentrations of benzoic acid and salicylic acid on quinoa plant increased growth, concomitantly with an increase in the levels of IAA, photosynthetic pigments, phenol, free amino acid contents, total soluble sugar, total carbohydrates and yield components. 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. Moreover application of benzoic acid or salicylic acid gave higher nutritional value of carbohydrate%, protein%, total flavonoids, phenol, antioxidant activity, in yielded seeds of quinoa plant

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