American-Eurasian Journal of Agronomy 12 (2): 19-25, 2019
ISSN 1995-896X

DOI: 10.5829/idosi.aeja.2019.19.25

Improving Growth and Productivity of *Quinoa* (*Chenopodium quinoa* Willd.) Grown under Sandy Soil Conditions via Exogenous Application with Some Antioxidant

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Abstract: Antioxidant substances could be used as a potential growth regulator, especially under environmental stress conditions. Thus, two field experiments were carried out during the two successive season (2015/2016 and 2016/2017) at the Research and production Production Station of National Research Centre; Al-Nubaria District; El-Behaira Governorate, Egypt to investigate the physiological role of three antioxidant (citric acid; ascorbic acid and ascobin at 100,200 and 300 ppm) in improving growth, productivity and quality of quinoa plant grown under sandy soil conditions. Data show that all applied treatments led to better performance of the plants expressed as increased growth, yield and its components as well as quality of seeds (carbohydrate and oil contents). Quinoa yield /feddan ranged between 300 and 564.2 kg/feddan according to the applied treatments. The most optimum treatment was ascobin at 200 ppm since it increased shoot dry weight /plant by 181.25% and root dry weight/plant by 172.97%, total photosynthetic pigments by 92.12%, IAA by 111%, seed yield/plant by 281.25%. There were gradual increases in seed yield/feddan as the level of antioxidant dose increased. Ascobin at 200 and 300 ppm as well as citric acid at 300 ppm resulted in seed yield increase > 80% over the untreated control which reflects the promotive effects of such antioxidants under such poor soils.

Key words: Ascobin · Ascorbic acid · Citric acid · Quinoa

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd) crop was chosen by FAO to be one of the important crops which play major role in food security assuring in the 21th century due to its high nutritional value and its good tolerance to adverse climatic conditions e.g. soil salinity, extreme pH, drought and frosts [1, 2] Moreover, quinoa is considered as a multipurpose crop because of the high-quality protein seeds, especially rich in essential amino acids, minerals, carbohydrates, antioxidant compounds as carotenoids, flavonoids, vitamin C and dietary fiber compared to that of cereals such as corn, oat, rice and wheat [3, 4]. In addition, [5] illustrated that quinoa seeds have enormous potential in the food industry as being gluten-free and highly nutritious did not contain anti-nutritional factors. Moreover, quinoa could be mixed with wheat in making bread [6].

Under environmental stress conditions, antioxidants could be used as a potential growth regulator in several plant species. Citric acid, ascorbic acid and ascobin act as antioxidant compounds when added in small quantities to plants, they react rapidly with radical intermediates of an auto-oxidation chain and stop it from progressing [7, 8].

Citric acid is considered to be as one of non-enzymatic antioxidants that eliminate free radicals produced in plants under stress [9] and induce defense mechanisms by increasing the activities of antioxidant enzymes [10].

Ascorbic acid plays several roles in plant growth, cell division, cell wall expansion [11], gene expression and other developmental processes as synthesis of many hormones and flavonoids [12]. In addition, ascorbic acid is a key substance in the network of plant antioxidant, that detoxify H_2O_2 to counteract oxygen radicals [13].

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Ascorbic and citric acids as natural organic antioxidants compounds have an auxinc action and synergistic effect on improving fruit retention [14, 15].

Ascobin (ascorbic acid and citric acid with ratio of 2:1), had a promotion effect on growth and active constituents compounds of various plants under normal or stressed conditions [16, 17]. Moreover, ascobin treatment stimulated growth parameters, endogenous growth hormones, carbohydrate constituents and wheat grain yield under normal and salinity conditions [17]. Since The quinoa crop is candidate to be grown in the reclamation areas with severe conditions, therefore this work was carried out to investigate the physiological role of some antioxidant in improving growth, productivity and quality of quinoa.

MATERIALS AND METHODS

Field experiments were conducted at the Experimental Station of National Research Centre, Nubaria district Beheira Governorate, Egypt, during two successive seasons (2015/2016 and 2016/2017). The soils of both experimental sites were sandy soil. The mechanical and chemical analysis of the experimental soil is reported in Table (1) according to [18].

Seeds of quinoa (*Chenopodium quinoa* Willd.) were obtained from Agricultural Research Centre Giza ; Egypt. The experimental design was Complete Randomized Block Design with four replicates. Quinoa seeds were sown on November 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).

Phosphatic fertilizer was applied pre-sowing at 150 kg/feddan as calcium super-phosphate (15.5% P_2O_5). 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 1^{st}, 2^{nd}, 3^{rd}, 4^{th} and 5^{th} irrigation. 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 5 days.

The plants were sprayed twice with citric acid, ascorbic acid and ascobin at different concentrations (100, 200 and 300 mg/l) during vegetative growth at 45 and 60 days after sowing, while control plants were sprayed with distilled water.

Data Recorded

Growth Characteristics: Plant samples were collected at 75 days from sowing to determine shoot height; fresh and dry weights of shoots and roots/plant as well as biochemical parameters in fresh leaves were determined as photosynthetic pigments, indole acetic acid contents and total phenolic content.

Yield and Yield Attributes: At harvest, the following traits were estimated: plant height, fruiting branches number /plant, weight of seeds/ plant, weight of shoot/ plant seed yield /feddan. Air dried seeds were ground into fine powder and kept in desiccators for analysis as determinations of carbohydrates and oil %.

Chemical Analysis: Photosynthetic pigments in fresh leaves were estimated using the method of [19]. Indole acetic acid content were extracted and analyzed by the method of [20]. Total phenolic content, the extract was extracted as IAA extraction and then measured as described by [21]. Determination of total carbohydrates was carried out calorimetric according to [22]. Determination of oil content was carried out according to [23].

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

A. Mechanical analysis:

Sand				
Course 2000-200µ%	Fine 200-20µ %	Silt 20-0µ%	Clay < 2µ%	Soil texture
47.64	36.59	12.66	4.18	sandy

B. Chemical analysis:

				Soluble Cations meq/l				Soluble anions meq/l			
pH 1:2.5	EC dSm^{-1}	$CaCO_3$	OM%	Na^+	K^+	Mg^+	Ca^{++}	CO_3^{-}	HCO_3^-	Cl^-	SO_4^{-}
7.60	0.13	5.3	0.06	0.59	0.14	0.95	1.0	0.0	1.27	0.46	0.87

Available nutrients

Macro element ppm		Micro element ppm				
N	P	K	Zn	Fe	Mn	Cu
51	12.2	74	0.13	1.3	0.28	0.00

Table 2: Effect of antioxidant on some growth parameters of quinoa plant grown under sandy soil conditions

Treatments	Shoot height (cm)	Fresh weight of shoot/plant (g)	Dry weight of shoot/plant (g)	Root length (cm)	Fresh weight of root/plant (g)	Dry weight of root/plant (g)
Control	34e	34.3f	3.68e	15f	1.59g	0.37e
Citric acid 100 ppm	52bc	81.9cd	9.37b	22.3bc	2.50f	0.38e
Citric acid 200 ppm	47cd	86.57c	10.42a	21c	6.30c	0.65b
Citric acid 300 ppm	46cd	102.35a	10.80a	23.5b	5.15d	0.65b
Ascorbic acid 100 ppm	36.67e	35.77f	4.04e	16.5def	1.72g	0.42de
Ascorbic acid 200 ppm	44d	58.85e	6.45d	16ef	2.44f	0.48cd
Ascorbic acid 300 ppm	48bcd	75.35d	8.89c	17.5de	2.98e	0.50c
Ascobin 100 ppm	46cd	93.35b	9.69b	21c	5.20d	0.51c
Ascobin 200 ppm	53.5b	86.15c	10.35a	27a	9.55a	1.01a
Ascobin 300 ppm	61a	77.55d	8.80c	18.5d	9.05b	0.94a

Table 3: Effect of antioxidant on photosynthetic pigments and some biochemical constituents of quinoa fresh leaf tissues

Treatments	Chlorophyll a	Chlorophyll b	Carotenoid	Total photosynthetic pigments	IAA µg/100g	Phenolic compound mg/100g
	----------mg/g----------					
Control	0.84e	0.21d	0.22bcd	1.27e	35.71i	194.45a
Citric acid 100 ppm	1.11cde	0.25cd	0.25bcd	1.62cd	42.97h	162.13b
Citric acid 200 ppm	1.22bcd	0.31bc	0.27bc	1.81bc	52.32f	159.36b
Citric acid 300ppm	1.31bc	0.32bc	0.28b	1.91bc	61.19d	145.55cd
Ascorbic acid 100 ppm	0.88e	0.23d	0.20d	1.31de	45.68g	151.06c
Ascorbic acid 200 ppm	0.93e	0.25cd	0.21d	1.39de	59.89e	142.73d
Ascorbic acid 300 ppm	0.99de	0.22d	0.23bcd	1.44de	63.65c	133.95e
Ascobin 100 ppm	1.25bcd	0.31bc	0.28b	1.83bc	52.64f	142.13d
Ascobin 200 ppm	1.65a	0.40a	0.39a	2.44a	75.35a	113.43f
Ascobin 300 ppm	1.45ab	0.34ab	0.25bcd	2.03b	72.32b	116.46f

Statistical Analysis: Data of the present study were subjected to the analysis of variance test (ANOVA) as Complete Randomized Block design (CRBD) according to [24], Since the data in both seasons took similar trends, Bartlett's test was applied and the combined analysis of the data was done. Treatments means were compared using [25] test at 5% of probability and presented with the standard errors.

Results and Discussion

Growth Parameters: Regarding vegetative growth parameters of quinoa (Table 2), it can be noted that all applied treatments (ascorbic acid at 200,300 ppm; citric acid and ascobin at 100,200,300 ppm) significantly increased shoot height, root length, fresh weight and dry weight of shoot and root/plant. The most optimum treatment was ascobin at 200 ppm since it increased shoot dry weight /plant by181.25 % and root dry weight/plant by 172.97% over control.

The positive effect of citric acid on growth parameters of quinoa plants may be attributed to the nature of citric acid as important substrate in Krebs cycle which plays an important role in stimulating biosynthesis processes [26, 27] reported that citric acid had stimulating effect on roots formation which encouraging the translocation of nutrients from soils via roots.

Meanwhile, the positive effect of ascorbic acid may be due to effective role of ascorbic acid in stimulating many physiological processes, such as respiration activities, cell division, cell enlargement and many enzymes activities as reported by [28-31].

Ascobin contains ascorbic and citric acid (natural organic antioxidants compounds) and has auxinic and promotion effect on growth and active constituents compounds on various plants [14, 15, 18, 32] mentioned that ascobin treatment stimulated wheat growth parameters under normal and salinity conditions.

Photosynthetic Pigments, Total Phenolic Content and Indole Acetic Acid: Table (3) shows that ascorbic acid at all doses did not cause significant increases in chlorophyll a, b, carotenoids and total photosynthetic pigments. On the other hand, all doses of citric acid and ascobin caused significant increases in all components of photosynthetic pigments. It is worthy to mention that ascobin treatment at any level were the most pronounced treatments especially at 200 ppm since it increased total photosynthetic pigments by 92.12%.

Table 4: Effect of antioxidant on yield and yield components of quinoa plant grown under sandy soil.

Treatments	Shoot height (cm)	Number of fruiting branches/plant	Plant weight (g)	Seed weight /plant (g)	Seed yield /fed.(kg)	* Relative yield %	Total carbohydrate %	Oil %
Control	39f	10e	10.05d	2.4d	300.0d	100	69.74c	5.72e
Citric acid 100 ppm	47e	14d	12.91cd	3.75c	340.9c	113.6	69.91c	6.49cd
Citric acid 200 ppm	51de	14.5d	13.90cd	4.70c	420.2b	140.1	71.14bc	7.23ab
Citric acid 300 ppm	71.17a	15.8cd	19.28b	6.03b	545.5ab	181.8	73.92a	7.36a
Ascorbic acid 100 ppm	54de	14.67d	11.00d	3.67cd	322.6cd	107.5	71.15bc	5.98de
Ascorbic acid 200 ppm	58cd	16.5cd	15.05c	4.70c	458.2b	152.7	72.43ab	6.62bc
Ascorbic acid 300 ppm	68.5ab	19.5ab	20.05b	4.30c	520.4ab	173.5	72.76ab	6.91abc
Ascobin 100 ppm	75a	14.5d	19.60b	9.05a	364.3cd	121.4	71.96ab	7.04abc
Ascobin 200 ppm	75.5a	20a	28.50a	9.15a	562.6a	187.5	72.55ab	7.36a
Ascobin 300 ppm	63.5bc	17.5bc	19.65b	6.40b	552.2a	184.1	71.98ab	7.45a

• Relative to the untreated control

The increases in photosynthetic pigments of quinoa plant in response to ascorbic acid treatment could be attributed to the role of ascorbic acid in regulating photosynthetic capacity by controlling stomatal movement [33]. Moreover, ascorbate is also an important co-factor of some enzymes or protein complexes that are involved in the regulation of photosynthesis [34] and scavenging reactive oxygen species [35].

The beneficial effect of ascobin on photosynthetic pigments may be due to its role in decreasing the rate of photochemical reduction, chloroplast structure and photosynthetic electron transfer [36]. Moreover, it is noticed that, carotenoids content was significantly higher in quinoa plants under all treatments. [37] reported that carotenoids play a role as a free radical scavenger which, enhance their capacity to reduce the damage caused by reactive oxygen species ROS, which in turn increased chlorophyll content of plants.

Regarding total phenolic content and indole acetic acid in quinoa fresh leaves, Table (3) shows that all applied treatments caused significant decreases in total phenolic content accompanied by significant increases in IAA when compared to control. Ascobin treatment at 200 ppm showed the highest significant increases in IAA by 111% over the control treatment.

It is worthy to mention that total phenolic compounds play a significant mechanism in regulation of plant metabolic processes [38, 39] and act as a substrate for many antioxidants enzymes [40].

Applications of ascorbic acid, citric acid and ascobin as foliar treatment at different concentrations on quinoa plants increased total indole acetic acid contents (Table 2). It is clear that the increase in auxin contents concurrent with the increase in growth rate as shown in Table (2). [41, 42] stated that ascorbic acid treatments increased IAA contents due to its effective role in stimulation of cell division and/or cell enlargement and subsequently growth.

Quinoa Yield and its Parameters: Table (4) shows that all applied treatments caused significant increases in shoot height, fruiting branches number and seed yield /plant as compared to control. Application of 200 ppm ascobin treatment was the most optimum treatments.

Data presented in Table 4 show that quinoa yield /feddan ranged between 300 and 564.2 kg/feddan according to the applied treatments. It is clear from the data the there were gradual increases in seed yield/feddan as the level of antioxidant dose increased. Ascobin at 200 and 300 ppm as well as citric acid at 300 ppm resulted in seed yield increase > 80% over the untreated control which reflects the promotive effects of such antioxidants under such poor soils. This increase in yield and its attributes may be due to the increase in growth parameters (Table 2) and photosynthetic pigments content (Table 3) and consequently stimulate many physiological processes. Moreover, the increase in yield and its components might be due to the effect of antioxidants role on enhancing different metabolic synthesis and delaying senescence. Citric acid is among the intermediate organic acids in Krebs cycle which produces cellular energy by oxidative phosphorylation [35]. Ascobin treatments had the most pronounced effect in comparison with other treatments. It may be due to that ascobin contain organic acids as ascorbic acid and citric acid with ratio of 2:1 that had a promation effect on growth and active constituents compounds on various plants because of their auxinic and synergistic effect on plants [18].

Nutritive Value of Seeds: Total carbohydrate content in the quinoa seeds was significantly increased by ascobin treatments at all doses and by ascorbic acid and citric acid at 200 and 300 ppm (Table 4). Moreover, all applied treatments significantly increased oil content of the yielded quinoa seeds except ascorbic acid at 100 ppm relative to control.

Similar finding were obtained in different plant species in response to ascorbic acid application where [43] found that total carbohydrate and protein concentrations were increased in faba bean plants. In addition, [44] found that ascorbic acid application increased oil percent in different flax cultivars.

[45] stated that ascobin have positive effect on the accumulation of essential oil yield and its percentage for rue plant and its ascorbic acid content induce many stimulating effects on physiological activities and its citric acid content is involved in Kerbs cycle [46, 36]. Moreover, the beneficial effect of citric acid on enhancing the biosynthesis of organic foods as well as its action as natural auxins [48]. Moreover, ascobin treatment stimulated the wheat growth parameters, endogenous growth hormones, carbohydrate constituents and grain yield under normal conditions and salinity levels (18).

CONCLUSION

Ascorbic acid, citric acid and ascobin at 100,200,300 ppm have stimulating effect on growth, productivity and quality of quinoa plants. Gradual increases in seed yield/feddan as the level of antioxidant dose increased. Ascobin at 200 and 300 ppm as well as citric acid at 300 ppm resulted in seed yield increase > 80% over the untreated control which reflects the promotive effects of such antioxidants under such poor soils.

ACKNOWLEDGEMENT

This work was funded by The National Research Centre through the project entitled "The Future of Agriculture and Using Some Promising Crops (Quinoa-Jojoba) Under Egyptian Conditions. Project No. 11030126 during 2016-2019.The principal investigator Dr/ Tarek Abdel Fattah Elewa-Field Crops Research Department, National Research Centre.

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