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Research Article
Physiological Role of Brassinosteroids and Cauliflower Extract on Quinoa Plant Grown under Sandy Soil

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Abstract
Background and Objective: Cauliflower extract is a source of brassinosteroids. Brassinosteroids are steroidal phytohormones which regulate plant growth, development and plant productivity. Thus, it could be used in improving growth and increasing plant yield under newly reclaimed sandy soil. **Materials and Methods:** Two field experiments were carried out, during 2 successive winter seasons to compare between the effect of brassinosteroids and 80% ethanolic extract of cauliflower buds on growth, some biochemical parameters, yield quantity and quality of quinoa plant grown under sandy soil conditions. **Results:** Results showed that brassinosteroids and 80% ethanolic extract of cauliflower significantly increased growth criteria of quinoa plant (plant height, branches and leaves number/plant, fresh and dry weight/plant), photosynthetic pigments (chlorophyll a, b, carotenoid and total photosynthetic pigments), seeds yield/plant, yield attributes (plant height, number of fruiting branches, dry weight of plant and 1000 seeds weight) as well as nutritional value of the yielded seeds (carbohydrate, oil, protein, proline and free amino acids contents as well as N, P, K content), antioxidant compounds (flavonoids and phenolic compound) and antioxidant activity. **Conclusion:** Cauliflower extract at different concentrations were more effective in increasing plant growth, quality and quantity of the yielded quinoa seeds than brassinosteroids treatments. The results indicated that cauliflower extract at 200 mg L^{-1} was the most pronounced treatment.

Key words: *Brassica oleracea* L., *Chenopodium quinoa* L., steroids hormones, antioxidant substance, seed quality

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd) seeds newly introduced multi-purpose crop because of its utilizing as human food, in flour products and in animal feedstock[1]. Quinoa seeds have high nutritive value; contain carbohydrates (77.6%), protein (12.9%), a balanced amino acid spectrum with high lysine, methionine contents and lipids (6.5%), dietary fibers, different vitamins and essential minerals, so quinoa could be used in bread and other seed products[2,3]. In addition, quinoa seeds take part in food industry as being gluten-free seeds and do not contain anti-nutritional factors[4]. Quinoa plant can grow in sandy soil of arid and semiarid regions under different abiotic stresses because of its salinity and drought tolerant[2]. It is necessary to increase quinoa productivity per unit area which could be achieved by selecting high yielding cultivars and improving the agricultural treatments as well as treated plant with natural compounds.

Recently, a great attention has been paid on the possibility of using natural safety substances to improve plant productivity and quality. Thus, development of safer natural antioxidants from extracts of spices and other plant materials that can replace synthetic antioxidants is of interest[5]. *Brassica* vegetables have been identified as an excellent source of antioxidants. Much research has focused on the antioxidant activity of the *Brassica* vegetables, especially cauliflower[6,7]. Koksal and Gulcin[6] reported that either water or ethanolic cauliflower extracts have powerful natural antioxidant activity against various synthetic antioxidant systems *in vitro*. Farooq *et al.*[7] mentioned that aqueous solvents (aqueous methanol (80% v/v) and aqueous ethanol (80% v/v)) were superior in their ability to extract the antioxidants of cauliflower than methanol and ethanol. They added that total phenolic content of cauliflower was 6.24 and 7.68%, antioxidant activity was 66 and 67% in aqueous ethanol and aqueous methanol, respectively. According to Velioglu *et al.*[8], a very positive relationship between total phenolics and antioxidant activity was found in many plant species. Fathima *et al.*[9] stated that cauliflower is a natural source of brassinosteroids and isopropyl cauliflower extract at 3% has significant effect on several metabolic processes, enhance *Gosssypium hirsutum* L. and *Vigna mungo* L. plant growth and development and photosynthesis process.

Brassinosteroids represent a class of plant hormones[10] that required for normal growth and development of plants and have crucial role under stress condition as reported[11,12]. Brassinosteroids stimulate a variety of physiological process including changes in enzymatic activities, membrane potential, photosynthetic activity, DNA, RNA, protein synthesis and the balance of other endogenous phytohormones[13]. Application of biological active brassinosteroids cause remarkable growth responses in plants, including stem elongation, pollen tube growth, leaf bending, leaf unrolling, xylem differentiations and regulation of gene expression[14]. Ryu *et al.*[15] proposed that brassinosteroids is critical for signaling in plant growth and development. Moreover, Brassinosteroids have dramatic pleiotropic effects on a broad range of diverse developmental pathways i.e., cell division and cell elongation, photomorphogenesis, reproductive development and leaf senescence[16,17]. Jiang *et al.*[18] mentioned that brassinosteroids have growth promoting properties and enhancing crop production. It has the ability to enhance the metabolic reactions by enhancing activity of some enzyme under seasonal condition[19].

The aim of this study was to study the physiological role of different concentrations of brassinosteroids (50, 100 and 150 mg L^{-1}) or 80% ethanolic extracts of cauliflower (100, 200 and 300 mg L^{-1}) on growth, photosynthetic pigments, seed yield and nutritional value of quinoa plant grown under sandy soil conditions.

MATERIALS AND METHODS

Study area: Two field experiments were conducted at the Experimental Station of National Research Centre, Nubaria district, Beheira Governorate, Egypt, during two successive winter seasons of 2016/2017 and 2017/2018. The soil of experimental site was sandy soil where mechanical and chemical analyses are reported by Chapman and Pratt[20] in Table 1.

Extraction procedure: Cauliflower buds were obtained from a local market in Cairo, Egypt. Whole cauliflower buds were cut into small pieces, ground in a blender with 80% ethanol and soaked for 24 h. Then, the extract was filtered over Whatman No. 1 paper. The residue was re-extracted under same conditions for 24 h. The filtrate was collected. Then, ethanol was removed using a rotary evaporator at 50°C. The extracts were placed in a dark bottle and stored at -20°C until used.

Experimental procedure: Quinoa seeds (Quinoa 1 cultivar) were obtained from Agricultural Research Centre Giza, Egypt. The experimental design was a complete randomized block design with 4 replicates. Quinoa seeds were sown on November of two successive seasons 2016/2017 and 2017/2018 at the rate of 60 kg/faddan (one faddan = 0.42 ha) in rows 3.5 m long and the distance between rows was

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

Sand				
Course 2000-200 μ (%)	Fine 200-20 μ (%)	Silt 20-0 μ (%)	Clay <2 μ (%)	Soil texture
Mechanical analysis				
47.64	36.59	12.66	4.18	Sandy

				Soluble cations (meq L^{-1})				Soluble anions (meq L^{-1})			
pH 1:2.5	EC (dS m^{-1})	$CaCO_3$	OM (%)	Na^+	K^+	Mg^+	Ca^{++}	CO_3^-	HCO_3^-	Cl^-	SO_4^-
Chemical analysis											
7.50	0.13	5.3	0.06	0.59	0.14	0.95	1.0	0.0	1.27	0.46	0.87

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

EC: Elemental carbon, OM: Organic matter

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 5 equal doses before the 1st, 2nd, 3rd, 4th and 5th irrigation. Potassium sulfate (48.52% K_2O) was added in two equal doses of 50 kg/feddan, before the 1st and 3rd irrigations. Irrigation was carried out using the new sprinkler irrigation system where water was added every 5 days. Quinoa plants undergo foliar spraying with brassinosteroids (0, 50, 75 and 100 mg L^{-1}) or ethanolic extract of cauliflower buds (0, 100, 200 and 300 mg L^{-1}) twice after 45 and 60 days from sowing.

Data recorded: Plant samples were taken after 75 days from sowing for determination of some growth parameters as plant height (cm), branches and leaves number/plant, fresh and dry weight of shoot/plant (g) and photosynthetic pigments of fresh leaf tissues. At maturity of plants, seed yield and its components as plant height (cm), number of fruiting branches/plant, dry weight of plant (g), seed yield/plant and 1000 seeds weight (g) were recorded. Nutritive value of the yielded seeds as total carbohydrates, oil, protein, proline, free amino acids, N, P, K contents as well as flavonoids, phenolic contents and antioxidant activities were determined.

Chemical analysis: Photosynthetic pigments (chlorophyll a, b and carotenoid) in fresh leaf tissues were estimated by using the method[21]. Total carbohydrate was extracted according to Herbert *et al.*[22] and determined colour imetrically according to DuBois *et al.*[23]. The oil content was extracted and determined[24]. Quinoa seeds were digested using an acid mixture consisting of nitric, perchloric and sulfuric acids in the ratio of 8:1:1 (v/v). Phosphorus and potassium were estimated photometrically using flame photometer method[20]. Total nitrogen (N) was determined using micro-Kjeldahl method[25]. Protein content was calculated by multiplying N (%) by 6.25. Proline and free amino acid contents were extracted according to the method[26]. Proline was assayed according to Bates *et al.*[27]. Free amino acids were determined with ninhydrin reagent method[28]. Total phenolic contents were extracted and determined according to Danil and George[29]. Flavonoid content was determined by the aluminium chloride colourimetric method[30]. Free radical scavenging activity (DPPH %) was done according to the method[31].

Statistical analysis: Data was statistically analyzed at 5% probability[31,32].

RESULTS

Changes in growth criteria: Data represents in Table 2 show the effect of brassinosteroids (50, 100 and 150 mg L^{-1}) and cauliflower ethanolic extract (100, 200 and 300 mg L^{-1}) on growth criteria of quinoa plant. All treatments significantly increased growth criteria of quinoa plant (plant height, branches and leaves number/plant, fresh and dry weight/plant) compared to control plants. It was noted that cauliflower ethanolic extract was more effective than brassinosteroids treatment as it caused more significant increases in different studied morphological parameters. It was noted that 200 mg L^{-1} cauliflower ethanolic extract was the most pronounced treatment (plant height, 43.66, branches number/plant 6.00, leaves number/plant 35.95, fresh weight/plant 69.25 and dry weight of plant 11.05).

Table 2: Effect of brassinosteroids (0, 50, 100 and 150 mg L^{-1}) or cauliflower ethanolic extract (0, 100, 200 and 300 mg L^{-1}) on growth criteria of quinoa plant grown under sandy soil conditions

Treatments	Concentration (mg L^{-1})	Plant height (cm)	Branches number/plant	Leaves number/plant	Fresh weight/plant (g)	Dry weight/plant(g)
Control	0	31.00	4.00	20.52	33.27	7.85
Brassinosteroids	50	36.66	5.33	24.52	49.52	9.11
	100	38.66	5.66	32.52	63.52	10.21
	150	35.66	5.00	33.52	52.52	9.74
Cauliflower	100	41.33	5.66	25.52	53.12	9.29
ethanolic extract	200	43.66	6.00	35.95	69.25	11.05
	300	39.00	5.00	33.52	61.52	9.89
LSD at 5%		1.68	0.94	1.77	2.15	0.75

Data are means of two seasons

Table 3: Effect of brassinosteroids (0, 50,100 and 150 mg L^{-1}) or cauliflower ethanolic extract (0, 100, 200 and 300 mg L^{-1}) on photosynthetic pigments (mg g^{-1} fresh weight) of quinoa plant grown under sandy soil conditions

Treatment	Concentration (mg L^{-1})	Chlorophyll a	Chlorophyll b	Carotenoids	Total pigments
Control	0	0.952	0.552	0.298	1.802
Brassinosteroids	50	1.241	0.612	0.324	2.177
	100	1.622	0.652	0.421	2.695
	150	1.532	0.642	0.374	2.548
Cauliflower ethanolic extract	100	1.302	0.624	0.342	2.268
	200	1.641	0.732	0.435	2.808
	300	1.520	0.712	0.395	2.627
LSD at 5%		0.06	0.04	0.03	0.07

Data are means of two seasons

Table 4: Effect of brassinosteroids (0,50, 100 and 150 mg L^{-1}) or cauliflower ethanolic extract (0, 100, 200 and 300 mg L^{-1}) on seed yield and yield attributes of quinoa plant grown under sandy soil conditions

Treatments	Concentration (mg L^{-1})	Plant height (cm)	Number of fruiting branches /plant	Dry weight/plant (g)	Seeds yield/plant (g)	1000 seed weight (g)
Control	0	51.67	12.67	16.74	4.85	1.06
Brassinosteroids	50	56.67	15.33	18.17	6.01	1.45
	100	66.67	20.67	23.12	8.67	1.98
	150	62.33	22.00	20.40	9.76	1.49
Cauliflower ethanolic extract	100	61.67	18.33	21.00	7.34	1.85
	200	68.50	24.33	29.67	11.32	2.35
	300	63.33	23.00	23.76	10.51	2.19
LSD at 5%		4.04	3.37	0.93	0.18	0.14

Data are means of two seasons

Changes in photosynthetic pigments: In response to foliar application with brassinosteroids and cauliflower ethanolic extract at different concentrations on quinoa leaves, photosynthetic pigments significantly increased as compared with control (Table 3). The magnitude of increase was more pronounced by ethanolic extract of cauliflower at different concentrations. The 200 mg L^{-1} cauliflower ethanolic extract was the most effective treatment followed by 100 mg L^{-1} brassinosteroids relative to control.

Changes in seed yield and yield attributes: Data presented in Table 4 show that brassinosteroids (50, 100 and 150 mg L^{-1}) or cauliflower ethanolic extract (100, 200 and 300 mg L^{-1}) significantly increased seed yield and yield attributes (plant height, number of fruiting branches/plant, dry weight of plant and weight of 1000 seeds) of quinoa plants compared with control plant. Foliar treatment with cauliflower ethanolic extract at different concentrations was more effective than brassinosteroids treatments. Moreover, the most effective treatment was 200 mg L^{-1} cauliflower ethanolic extract as compared with the other treatments.

Changes in nutritional value of the yielded seeds: The presented data in Table 5 show the effect of brassinosteroids (50, 100 and 150 mg L^{-1}) or cauliflower ethanolic extract (100, 200 and 300 mg L^{-1}) on nutritional value of the yielded quinoa seeds. The results show that brassinosteroids and cauliflower ethanolic extract treatments significantly increased the nutritional value of the yielded seeds (carbohydrates, oil, protein, proline and free amino acids contents as well as nitrogen, phosphorus and potassium contents) as compared to control plants. It was noted that ethanolic extract of cauliflower treatments were more effective than

Table 5: Effect of brassinosteroids (0,50, 100 and 150 mg L^{-1}) or cauliflower ethanolic (0, 100, 200 and 300 mg L^{-1}) on nutritional value of the yielded quinoa seeds

Treatments	Concentration (mg L^{-1})	Carbohydrate (%)	Oil (%)	Protein (%)	mg/100 g dry wt Proline	mg/100 g dry wt Free amino acids	mg/100 g dry wt N	mg/100 g dry wt P	mg/100 g dry wt K
Control	0	62.70	5.83	13.15	24.10	219.63	224.00	254.13	364.1
Brassinosteroids	50	63.87	6.37	14.08	32.54	264.42	238.77	274.03	386.83
	100	64.70	7.02	15.24	41.64	320.42	243.84	309.05	404.78
	150	63.48	7.05	14.65	35.60	288.52	234.34	291.87	382.24
Cauliflower ethanolic extract	100	64.47	6.49	14.33	34.30	274.30	229.28	287.58	401.12
	200	65.06	7.24	15.83	45.47	333.18	253.33	323.58	416.37
	300	63.67	6.91	14.83	38.71	296.05	237.33	302.92	409.15
LSD at 5%		0.57	0.16	0.25	0.31	2.97	7.09	4.15	2.09

Data are means of two seasons

Table 6: Effect of brassinosteroids (0, 50,100 and 150 mg L^{-1}) or cauliflower ethanolic extract (0, 100, 200 and 300 mg L^{-1}) on antioxidant substances and antioxidant activity of the yielded quinoa seeds

Treatments	Concentration (mg L^{-1})	Flavonoids (mg/100 g)	Phenolic contents (mg/100 g)	DPPH (%)
Control	0	32.00	97.73	32.90
Brassinosteroids	50	36.22	109.80	41.74
	100	40.62	123.47	45.64
	150	37.93	114.15	42.88
Cauliflower ethanolic extract	100	37.36	110.31	43.51
	200	45.75	128.38	48.82
	300	41.33	115.78	45.17
LSD at 5%		0.97	0.94	2.76

Data are means of two seasons

brassinosteroids treatments. Moreover, 200 mg L^{-1} cauliflower ethanolic extract was the most effective treatment over the rest of treatments.

Changes in antioxidant contents and antioxidant activity: All applied treatments significantly increased antioxidant compounds (flavonoids and phenolic contents) and antioxidant activities of the yielded quinoa seeds as compared to control (Table 6). It was noted that 200 mg L^{-1} cauliflower ethanolic extract was the most pronounced treatments followed by 100 mg L^{-1} brassinosteroids.

DISCUSSION

It is clear that brassinosteroids (50, 100 and 150 mg L^{-1}) and cauliflower ethanolic extract (100, 200 and 300 mg L^{-1}) significantly increased plant growth and yielded seed quality and quantity of quinoa plant grown under sandy soil conditions. These results are in a good agreement with those reported by Fathima *et al.*[9] and Nirmal *et al.*[33]. It is worthy to mention that cauliflower is a natural source of brassinosteroids as mentioned[9]. Zhang *et al.*[34] observed that brassinosteroids application improved assimilation of carbon and nitrogen by the stabilization of membrane structures and also improved plant growth, productivity and photosynthesis. Brassinosteroids is involved in a range of developmental processes, including cell division and elongation, vascular differentiation, reproductive development and modulation of gene expression[10]. Moreover, Mahesh *et al.*[35] and Bakry *et al.*[36] stated that treating radish seeds and flax plant with 24-epibrassinolide and stigmasterol increased growth parameters of radish plant and flax plants. They attributed the promotive role of brassinosteroids treatment on soluble sugar levels which is necessary for the turgor and increasing the efficiency of water uptake and protecting the photosynthetic pigments. Brassinosteroids act as growth stimulants which may play a role in enhancing quinoa plant growth and development via improving certain metabolic activities[37]. Different concentrations of brassinosteroids and cauliflower ethanolic extract significantly increased photosynthetic pigments of quinoa plant (Table 3). These results are in a good agreement with study reported by Cao and Zhao[38] they stated that spraying rice seedlings with brassinosteroids caused highly significant increases in photosynthetic pigment content of plants. Likewise, Abdel Hamid[39] and Filova *et al.*[40] found that brassinoloids treatments significantly increased photosynthetic pigments in wheat and sunflower plants as compared with control plants. Bajguz and Hayat[41] stated that brassinosteroids can regulate the initial carboxylation activity of ribulose 1,5-bisphosphate by influence photosynthetic CO_2

assimilation which is determined by electron transport efficiency in photosynthesis. It is stated that exogenous application of brassinosteroids has the ability of increasing the capacity of carbon oxide assimilation in Calvin cycle using increased initial activity of enzymes, such as RuBisCo, nitroreductase and glutamine synthesis[40,42].

Yield is a result of the integration of metabolic reactions in plants, consequently, any factor that influences this metabolic activity at any period of plant growth can affect the yield. Thus, in the present investigation all applied treatments caused significant increases in seed yield and its components (Table 4). In this connection, Bakry *et al*.[36] and Hashem *et al*.[43] found that stigmasterol treatments significantly increased flax yield and its components and attributed these increasing to the increment of growth regulators (as IAA) which improved photosynthetic activities and consequently had beneficially effects on the number and weight of capsules and seed yield. The promotive effect of different treatments of brassinosteroids and cauliflower ethanolic extract on yield and yield components of quinoa plant (Table 4) resulted from increased growth parameters (Table 2), photosynthetic pigments (Table 3) which increased levels of photo assimilates (Table 5, 6) and their translocation from leaves (source) to seeds (sink).

Exogenous application of brassinosteroids and cauliflower ethanolic extract significantly increased nutritional value of the yielded seeds of quinoa plant as it increased carbohydrate, oil, protein, proline and free amino acids contents as well as N, P and K contents (Table 5). Regarding carbohydrates contents of the yielded seeds, Hassanein *et al*.[44] confirmed that carbohydrate contents of faba bean plant was increased by using stigmasterol treatment. With respect to oil contents, Hashem *et al*.[43] and Bekheta *et al*.[45] concluded that application of stigmasterol, which exerts their effect on the level of gibberellins metabolism, might increase the accumulation of oil of thyme and flax plants respectively. Proline and free amino acids content were accumulated in quinoa seeds treated with all treatment (Table 5). In agreement with our findings, there is a report revealing an increase in the proline content following application of brassinosteroids (28-HBL and 24-EBL) in sorghum and flax plants[46,47].

Moreover, Table 6 showed the improving effect of all applied treatments on antioxidant substances and antioxidant activities of the yielded quinoa seeds. The increases in the scavenging activity could be attributed to the increases in total phenolics and total flavonoids[48].

Special attention must be paid to cauliflower extract benefits. Since, cauliflower treatments were more effective than brassinosteroids treatments. Besides cauliflower extract act as a natural source of brassinosteroids[9], it also characterized by considerable content of antioxidant substances (flavonoid and phenolic content)and antioxidant activity (PPH%) as mentioned by Koksal and Gulcin[6] and Farooq *et al*.[7]. Antioxidants may be defined as compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions[8]. Phenolic contents are important protective components of plant cells. The potential of phenolics to act as an antioxidant is mainly due to their properties to act as hydrogen donators, reducing agents and quenchers of singlet[49] O_2. Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic oxidative enzymes and anti-inflammatory. The phenolic compounds retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food[50]. It is well documented that application of natural substances extracted from *Brassica* plant on different plant species have pronounced effect in improving plant growth, productivity and yield quality either under normal conditions or stressed conditions[9,51-55].

The results of this article are very good for implementation on a large scale in the new lands such newly reclaimed sandy lands which suffers from bad environmental conditions such lack of water retention resulted by high speed of evaporation due to high temperatures or filtration speed. Application of sustainable farming methods are safer to the environment, the used treatments cauliflower extract and brassinosteroids are of these compounds as they increased growth and yield of quinoa plant via enhancing different physiological and biochemical processes so improving yield quantity and quality. The cauliflower ethanolic 200 mg L^{-1} extract was the most effective concentration.

CONCLUSION

It is recommended the use cauliflower ethanolic extract as a source of brassinosteroids hormone as a new natural and low-cost treatment for stimulating the growth with no discernible adverse effects and increase production of quinoa plant yield as well as improve quality of the yielded seeds.

SIGNIFICANCE STATEMENT

This study discovers the possible beneficial role of cauliflower ethanolic extract (as a natural source of brassinosteroids) or brassinosteroids which can be effective for improving growth and consequently yield quantity and quality of quinoa plant under sandy soil conditions. This study will help in increasing yield of different crops under sandy soil conditions to decrease the gap of food.

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