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Effect of foliar spraying with amino acid and cytokinin on growth, yield quality and quantity and nutritional status of roselle plants

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At two successive season (2016 and 2017), the present research has been made to study the effect of five rates of amino acids (0, 250, 500, 750 and 1000 ppm) combination with three rates of cytokinin (0, 20 and 60 ppm) on growth, yield quality and quantity and nutritional status of roselle plants (*Hibiscus sabdariffa* L.).The results indicated that increasing amino acid and cytokinin rates led to increasing growth and yield parameters. The high values of growth components (plant height, number of branches, dry weight of leaves and leaf area) and yield parameters (seed yield, seed yield per plant, fixed oil per plant and fixed oil yield) could be obtained under high level of amino acid (1000 ppm) and combined with third level of cytokinin (60 ppm).These higher values of growth components were187.8cm, 22.27, 50.87 g and 41.88 cm^2 of plant height, number of branches, dry weight of leaves and leaf area, respectively. As well as higher values of yield components were 10.49ml $plant^{-1}$, 136.3 l fed^{-1}, 50.32 g and 654.2 kg fed^{-1} of fixed oil $plant^{-1}$, fixed oil yield, seed yield $plant^{-1}$ and seed yield, respectively. The high levels of amino acids and cytokinin, which improved growth and yield, were the same as those that improved yield quality and some nutrients content. The highest rate of amino acid (1000 ppm) with higher rate of cytokinin (60 ppm) were given high values of N, P and K of leaves content (2.923, 0.490 and 1.193 %, respectively). As well as the same highest rates of amino acid and cytokinin given high values of anthocyanin, vitamin C and carbohydrate (270.2 mg 100 g^{-1}, 1.149 g $plant^{-1}$ and 41.28 %, respectively).

Keywords:Roselle plants, Amino acid, Cytokinin, growth, yield quantity and quality, Nutritional status

INTRODUCTION

Roselle *(Hibiscus sabdariffa* L.) is an annual herbaceous sapling pertinence to the malvaceae family. It is grown in orbital climates for its fleshy calyx and leaves (Bahaeldeen et al., 2012). In Egypt, India, Mali, Malaysia, Nigeria and Sudan, the plant was reported to contain high quantities of protein, lipids, organic acid, vitamins and nutrients (Fatoumata et al., 2011; Balogun and Olatidoye, 2012). It is known commonly as "karkade" in Egypt and most Arab countries (Mohamed et al., 2007).

Amino acids is a well-known biostimulant which has positive effects on growth, quality and quantity of yield at all plants and safely alleviates the injuries caused by abiotic stresses (Kowalczyk and Zielony 2008). Amino acids can influence the physiological activities in plant healthy growth such as exogenous application of amino acids have been reported to adjust the growth, production and quality of tomato in greenhouse (Boras et al., 2011). Saeed et al., (2005) found

that treatments of amino acids significantly increased growth components such as fresh weight of shoot and root as well as pod yield of soybean. Liu Xing et al.,(2008)indicated that foliar application of amino acid to radish plants increased N and P content of shoots. El-Zohiri and Asfour (2009) found that spraying of amino acids at 0.25 ml l^{-1} significantly increased vegetative growth expressed as plant height and dry weight of potato plant. Cytokinins were plant hormones known to be key regulators of various aspects of plant growth and development, including cell division, leaf senescence, apical dominance, lateral root formation, stress tolerance, and nutritional signaling (Sakakibara, 2006 and Argueso et al., 2009). Exogenous application of synthetic cytokinins, such as 6-benzylaminopurineand *N*-(2-chloro-pyridin-4-yl)-*N'*-phenylurea, could induce fruit set and development in fruit crops such as grape, kiwi fruit, melon, watermelon, apple, and pear (Stern et al., 2003, Kim et al., 2006 and Zabadal and Bukovac, 2006). Furthermore, endogenous levels of cytokinins have been linked with fruit growth (Srivastava and Handa, 2005). Therefore, cytokinins may play important roles in fruit development, but the mechanisms and regulation of their activity have not been well investigated.
The paper aimed to study the effects of different rates of amino acid and cytokinin on growth, yield quantity and quality and nutritional status of roselle plant in two consecutive seasons.

MATERIALS AND METHODS

A field experiment was conducted during two successive seasons 2016 and 2017 at the private farm in El-Behira governorate, to study the effect of five rates of amino acids in combination with three rates of cytokinin on growth, yield quality and quantity and nutritional status of roselle plants (*Hibiscus sabdariffa* L.). The applied rates of amino acids were 0, 250, 500, 750 and 1000 ppm and the rates of cytokinin were 0, 20 and 60 ppm. Soil of field experiment was sampled before planting, to determine the chemical properties according to the standard procedures outlined by Cottenie (1980) and are scheduled in Table (1).

Table (1): Some chemical properties of soil used

Soil property	Value
pH (1:2.5 soil suspension)	7.80
EC (dS m^{-1}), soil paste extract	1.16
$CaCO_3$ (%)	1.40
Soluble ions (mmol L^{-1})	
Ca^{++}	3.04
Mg^{++}	5.80
Na^{+}	2.45
K^{+}	0.33
CO_3^{--}	nd*
HCO_3^{-}	1.10
Cl^{-}	1.06
SO_4^{--}	9.46

Field experiment was arranged as factorial experiment (two way) based on randomized complete block design with three repetitions. The main plots assigned to amino acids and the sub plots deviated to cytokinin. After month from planting the applications were started and continue at 14 days intervals until the beginning of fruit maturity.

A sample of nine plants from every treatment were collected to measure the following growth parameters: plant height (cm), number of branches, dry weight of leaves (g) and leaf area (cm^2); and the following yield parameters: fixed oil $plant^{-1}$(ml $plant^{-1}$), fixed oil yield (l fed^{-1}), seed yield (g $plant^{-1}$) and seed yield (kg fed^{-1}).

Plant samples were oven dried at 65 C° for 48 hrs then ground and wet digested using H_2SO_4: H_2O_2 method described by Cotteine (1980).The digests were then subjected to measurement of N using micro Kjeldahl method; P was assayed using molybdenum blue method while K was evaluated by flame photometer (Chapman and Pratt, 1961). Vitamin C was determined in sepals according to the method described by A.O.A.C. (1980). Total anthocyanin content was measured in dried sepals according to the methods of DU and Francis (1973). Total carbohydrate percentage was quantified according to the method of Herbert et al., (1971). The percentage of fixed oil in seeds was estimated according to the method mentioned by A.O.A.C. (1980), and then fixed oil content per plant and per Feddan were calculated.

All data obtained were subjected to analysis of variance according to Snedicor and Cochran, (1980). The least significant differences (LSD) at P = 0.05 level was to verify the difference between means of the treatments.

RESULTS

Data in Table (2) showed to the main effect of amino acid rates (0, 250, 500, 750 and 1000 ppm) on growth and yield parameters of roselle plants. Increasing amino acid rates increased significantly growth parameters (plant height, number of branches, dry weight of leaves and leaf area) from

142.5 to 173.9 cm, 9.84 to 14.86, 20.48 to 32.45 g and 23.66 to 30.83cm^2, respectively. Therefore, the amount of increase in plant height, number of branches, dry weight of leaves and leaf area due to the increase in the rate of amino acids were 18.1, 33.8, 36.9 and 23.3 %, respectively. As well as when amino acid rates increased all yield parameters of Roselle plants (fixed oil plant^{-1}, fixed oil yield, seed yield plant^{-1} and seed yield from 2.667 to 5.307ml plant^{-1}, 34.77 to 68.93l fed^{-1}, 25.99 to 34.55 g and 297.9 to 449.2kg fed^{-1}, respectively) were significantly increased. Therefore, the amount of increase in fixed oil plant^{-1}, fixed oil yield, seed yield plant^{-1} and seed yield were 44.7, 49.6, 24.8 and 33.7%.

Amino acids were important for stimulation of cell growth, because it were contain both acid and basic groups and act as buffers, which helped to maintain favorable pH value within the plant cell (Rai, 2002). Also, amino acids were can act as parts of co-enzymes or as precursors of certain plant hormones and improve plant growth via improving photosynthesis (Amin et al., 2011). The regulatory effect of amino acids on plant could be explained by the notion that amino acids can affect plant growth and development through their influence on gibberellins biosynthesis (Satyanarayana et al., 2002). Also, amino total as a source of amino acids may be playing an important role in plant metabolism and protein assimilation which necessary for cell formation and consequently increase fresh and dry mater(Abo Sedera et al., 2010).

Data in Table (3) showed to the main effect of cytokinin rates (0, 20 and60 ppm) on growth and yield parameters of roselle plants. Increasing cytokinin from 0 to 60 ppm rates increased significantly growth parameters (plant height, number of branches, dry weight of leaves and leaf area from 156.2 to 175.9 cm, 12.08 to 18.79, 26.66 to 38.93 g and 26.49 to 35.17 cm^2, respectively) and increased significantly yield parameters (fixed oil plant^{-1}, fixed oil yield, seed yield plant^{-1} and seed yield from 3.276 to 7.610 ml plant^{-1}, 43.38 to 98.95l fed^{-1}, 29.13 to 41.42 g and 378.7 to 538.4kg fed^{-1}) of roselle plants. The amount of increase in plant height, number of branches, dry weight of leaves and leaf area due to the increase in the rate of cytokinin were 11.0, 35.7, 31.5 and 24.7 %, respectively, as well as the amount of increase in fixed oil plant^{-1}, fixed oil yield, seed yield plant^{-1} and seed yield were 57.0, 56.2, 29.7 and 29.7 %, respectively.

The catalytic effect of cytokinein is due to the role of cytokinins in increasing cell division in apical meristems and cambium and thus enhancing all growth parameters of plants, as well as the increment in branches number as a result of cytokinin application may be attributed to its influence on counteracting or eliminating the apical dominance (Azza et al., 2011). Abd El-Rheem and Mahdy (2014)showed that the effects of different levels of cytokinin on wheat growth and yield under different levels of nitrogen fertilization. They pointed out that increasing cytokinin level under different levels of nitrogen fertilization increasing grain, straw yield and some growth properties (such as leaves – and length and height plants) of wheat plants. The addition of cytokinin improved the yield of wheat despite the decreasing in the addition rate of nitrogen fertilizer; where the yield has not decreased much in the low rates of nitrogen fertilizer combined with high rate of cytokinin. Cytokinins have been shown to participate in the regulation of numerous aspects of plant development including initiation of buds, flowering, abscission and yield by enhancing the cell expansion.

The results in Table (4) presented the di-interaction effect between several rates of amino acid and cytokinin on growth and yield parameters of roselle plants. Increasing amino acid rates from 0 to 1600 ppm at 0 rate of cytokinin rates increased growth parameters (plant height, number of branches, dry weight of leaves and leaf area) and yield parameters (seed yield, seed yield per plant, fixed oil per plant and fixed oil yield) of roselle plants. The significant increase in values growth and yield parameters were evident at the high level of amino acid. On the other hand, increasing cytokinin levels up to third level (60 ppm) increased significantly all growth and yield parameters of Roselle plant under all amino acid levels. The highest values of growth components (plant height, number of branches, dry weight of leaves and leaf area) and yield parameters (seed yield, seed yield per plant, fixed oil per plant and fixed oil yield) could be obtained under highest level of amino acid (1000 ppm) and third level of cytokinin (60 ppm). Where growth components values reached 187.8cm, 22.27, 50.87 g and 41.88 cm^2 of plant height, number of branches, dry weight of leaves and leaf area, respectively. As well as higher values of yield components were 10.49ml plant^{-1}, 136.3 l fed^{-1}, 50.32 g and 654.2 kg fed^{-1}

Table (2): Main effect of amino acid rates on growth and yield parameters of roselle plants (the average of the two successive growing seasons).

Amino acid ppm	Plant height	No. of branches	Dry weight of leaves	Leaf area	Fixed Oil plant^{-1}	Fixed oil yield	Seed yield plant^{-1}	Seed yield
	cm		g	cm^2	ml plant^{-1}	l fed^{-1}	g	kg fed^{-1}
0	142.5	9.84	20.48	23.66	2.667	34.77	25.99	297.9
250	157.7	13.11	27.50	27.54	3.916	50.92	30.53	396.9
500	169.5	14.21	29.88	28.21	4.590	59.69	32.34	421.4
750	172.7	14.86	32.40	30.83	5.105	67.21	34.25	445.4
1000	173.9	14.15	32.45	30.68	5.307	68.93	34.55	449.2
L.S.D.$_{0.05}$	1.837	0.322	1.255	1.019	2.446	2.873	0.745	9.376

Table (3):Main effect of cytokinin rates on growth and yield parameters of roselle plants (the average of the two successive growing seasons).

Cytokinin ppm	Plant height	No. of branches	Dry weight of leaves	Leaf area	Fixed Oil plant^{-1}	Fixed oil yield	Seed Yield plant^{-1}	Seed yield
	cm		g	cm^2	ml plant^{-1}	l fed^{-1}	g	kg fed^{-1}
0	156.2	12.08	26.66	26.49	3.276	43.38	29.13	378.7
20	170.9	16.44	32.61	32.29	5.346	69.48	34.95	454.3
60	175.6	18.79	38.93	35.17	7.610	98.95	41.42	538.4
L.S.D.$_{0.05}$	1.102	0.193	0.753	0.611	1.468	1.724	0.447	5.626

Table (4): Di- interaction of amino acid and cytokinin on growth and yield components of roselle plants (the average of the two successive growing seasons).

Amino acid	Cytokinin	Plant height	No. Of branches	Dry weight of leaves	Leaf area	Fixed Oil plant^{-1}	Fixed oil yield	Seed Yield plant^{-1}	Seed yield
ppm		cm		g	cm^2	ml plant^{-1}	l fed^{-1}	g	kg fed^{-1}
0	0	108.4	5.650	15.18	18.52	1.327	17.19	20.15	261.9
	20	159.4	12.900	22.83	26.61	3.488	45.28	29.12	378.6
	60	164.4	13.43	26.53	28.70	3.786	49.21	31.33	407.3
250	0	158.7	12.26	24.79	25.15	3.256	42.35	28.45	369.9
	20	164.9	16.00	31.73	31.65	4.592	59.68	34.09	443.2
	60	166.2	17.43	33.28	33.08	5.996	78.06	36.30	471.9
500	0	165.7	13.40	29.03	27.64	3.671	47.69	30.49	396.3
	20	173.4	15.90	32.90	31.89	4.965	64.57	34.78	452.1
	60	176.5	19.77	38.26	32.52	8.293	107.9	42.32	550.1
750	0	173.8	14.70	31.15	31.04	4.017	56.54	33.72	438.4
	20	176.4	18.28	37.51	34.31	6.304	81.96	36.56	475.3
	60	183.3	21.04	45.69	39.67	9.485	123.3	46.82	608.7
1000	0	174.7	14.40	33.15	30.09	4.111	53.15	32.86	427.2
	20	180.2	19.10	38.08	37.01	7.380	95.94	40.19	522.5
	60	187.8	22.27	50.87	41.88	10.49	136.3	50.32	654.2
L.S.D.$_{0.05}$		4.108	0.721	2.807	2.280	0.547	6.425	1.667	20.97

The positive effect of amino acids on good growth of plants can be led to, the role of amino acids in maintaining the structure of proteins required for cell division, amino acids were helped plant cells to divide and enlarge by entering the hormone structures. As well, the ability of amino acids to be converted to polyamines which are effective in cell division, differentiation and growth. Further, amino acids can be effective by entering biological cycles like citric acid (Kakkar et al., 2000 and Heldr and Piechulla, 2010).

The main effect of amino acid and cytokininon quality of yield and nutrients content (N, p and K) of roselle plants is presented in Tables (5) and (6). Increasing amino acid levels from 0 to 1000 ppm or cytokinin levels from 0 to 60 led to increasing significantly N, P, K, anthocyanin, vitamin C and carbohydrate content of Roselle plants. It is also noted that to reach high values forN, P , K, anthocyanin, vitamin C and carbohydrate content, both amino acid and cytokinin should be in the higher rates (1000, 60 ppm , respectively).

Enhancing of the production of anthocyanin was high when cytokinin rates increased because anthocyanin serves as a hydroxyl radical scavenger(Cooper, 2001). Ahmed and Walid

(2016) found that using amino acid at 1000 ppm enhancing growth and yield components of Roselle plants such as plant height, number of branches, seed yield and fixed oil yield. The regulatory effect of amino acids on growth could be explained by the notion that some amino acids e.g. phenylalanine can affect plant growth and development through their influence on gibberellins biosynthesis.

The di-interaction effect of amino acid and cytokinin on yield quality and nutritional status of Roselle plants is presented in Table (7). Increasing amino acid rates from 0 to 1000 ppm increased significantly N, P, K, anthocyanin, vitamin C and carbohydrate content of Roselle plants under all rates of cytokinin. The highest rate of amino acid (1000 ppm) with highest rate of cytokinin (60 ppm) were given high values of N, P and K of leaves content (2.923, 0.490 and 1.193 % ,respectively). As well as those rates given high values of anthocyanin, vitamin C and carbohydrate (270.2 mg 100 g^{-1}, 1.149 g $plant^{-1}$ and 41.28 %, respectively).

The increase in photosynthetic pigments may be due to the incorporation of amino acids in anabolic processes and also due to enhancements of metabolic efficiencies in the plant (Bahari et al., 2013). Doaa and El-Aila (2015) reported that spraying amino acid at rate 750 ppm led to increasing N, P and K content of radish leaves.Janowska (2014) found that spraying of cytokinin at concentrations (100, 350 and 600 mg/dm^3) on calla lily resulted in increasing 2-3 times the flower yield, staying neutral towards flower quality and showed higher greenness index and protein content. Ramtin et al., (2015) indicated that the spraying cytokinin (benzyladenine) at 50 µM on carnation plants led to good improvement to water content, dry weight, total length, diameter and length floret of before harvesting. Matter (2016) showed that spraying cytokinin (100 mg L^{-1}) on roselle plants led to increase in chlorophyll a, b, carotenoids and anthocyanin, as well as the same concentration of cytokinin helped on increasing total carbohydrate by 11.76% and 7.71%, in the first and second seasons, respectively.

Table (5): Main effect of amino acid rates on yield quality and nutritional status of roselle plants (the average of the two successive growing seasons).

Amino acid ppm	Anthocyanin $mg100g^{-1}$	Vitamin C g $plant^{-1}$	Carbohydrate	N	P	K
			%			
0	85.37	0.738	26.38	1.591	0.305	0.632
250	116.2	0.809	31.07	1.955	0.359	0.804
500	137.9	0.855	32.67	2.083	0.378	0.863
750	156.3	0.888	33.55	2.110	0.391	0.908
1000	157.2	0.890	32.34	2.087	0.377	0.851
$L.S.D._{0.05}$	2.840	0.007	0.406	0.171	0.007	0.073

Table (6): Main effect of cytokinin rates on yield quality and nutritional status of roselle plants (the average of the two successive growing seasons).

Cytokinin ppm	Anthocyanin $mg100g^{-1}$	Vitamin C g $plant^{-1}$	Carbohydrate	N	P	K
			%			
0	112.1	0.789	30.48	1.795	0.348	0.773
20	168.4	0.901	36.63	2.349	0.425	1.001
60	211.2	0.998	38.28	2.592	0.453	1.090
$L.S.D._{0.05}$	1.704	0.004	0.244	0.103	0.004	0.044

Table (7): Di-interaction effect of amino acid and cytokinin on yield quality of roselle plants (the average of the two successive growing seasons).

Amino acid	Cytokinin	Anthocyanin	Vitamin C	Carbohydrate	N	P	K
ppm		mg100g^{-1}	g plant^{-1}	%			
0	0	59.06	0.537	19.59	1.140	0.23	0.407
	20	107.9	0.832	30.75	2.040	0.36	0.810
	60	118.0	0.874	32.15	2.110	0.387	0.860
250	0	96.84	0.805	29.88	1.773	0.333	0.757
	20	146.7	0.848	36.07	2.193	0.420	0.970
	60	169.9	0.876	37.76	2.470	0.440	1.05
500	0	119.7	0.845	33.21	1.873	0.377	0.837
	20	162.9	0.879	37.06	2.353	0.430	1.010
	60	231.7	1.007	39.83	2.637	0.470	1.147
750	0	140.1	0.886	34.93	1.993	0.400	0.947
	20	196.8	0.939	39.02	2.497	0.450	1.097
	60	266.0	1.084	40.36	2.820	0.480	1.200
1000	0	144.9	0.872	34.79	2.193	0.400	0.917
	20	227.7	1.007	40.26	2.663	0.460	1.117
	60	270.2	1.149	41.28	2.923	0.490	1.193
L.S.D.$_{0.05}$		6.349	0.016	0.908	0.381	0.016	0.016

CONCLUSION

Spraying with amino acids and cytokinin greatly enhances the growth, yield quantity and quality, as well as the nutritional status of roselle plants. The best experimental treatment to give the best results were spraying plants at 1000 ppm for amino acid mixed with 60 ppm cytokinin**.**

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All authors contributed equally in all parts of this study.

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