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Influence of L-ascorbate on yield components, biochemical constituents and fatty acids composition in seeds of some groundnut (*Arachis hypogaea* L.) cultivars grown in sandy soil

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Groundnut is one of the most cultivated oil crops in the world. Green house pot experiments were conducted in summer seasons of 2014 and 2015 to find out the influence of L-ascorbate (LAA) on improving yield components and biochemical constituents, as well as oil composition in harvested seeds of some groundnut cultivars (NC, Giza-6 and Gregory) grown in sandy soil. Results showed that, foliar application of LAA at different concentration (0, 400 and 600 mg/l) increased yield components including number of pods/plant, pods weight/plant (g), number of seeds/plant, seeds weight/plant (g), 100-seed weight (g) and oil yield/plant (g), as well as biochemical constitutes such as total free amino acids, total protein, mineral contents (nitrogen, phosphorous, potassium, zinc, manganese and iron), oil content and unsaturated fatty acids, but decreased saturated fatty acids in yielded seeds compared to control plants. Also, results reveal that total soluble sugars, total carbohydrates and total phenols were decreased in NC cv., meanwhile, these attributes were increased in Giza 6 and Gregory cultivars in response to application of LAA compared to untreated plants. The physiological responses of groundnut plants depended on the concentration of LAA and type of cultivar. In NC cv., the application of LAA at low concentration (400mg/l) seemed to be more effective than the higher one. On the contrary, in Giza-6 and Gregory cultivars, LAA at 600 mg/l seemed to be more effective than the lower concentration. Overall, foliar application of LAA has a positive role in enhancement the productivity and the biochemical constituents and fatty acids composition of groundnut cultivars (NC, Giza 6 and Gregory) grown in sandy soil.

Keywords: L-ascorbate, Groundnut, Sandy soil, Yield, Biochemical constituents, Fatty acids composition.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an important edible oil seed crop in world (FAO, 2012). The groundnut has an important role in the economy of several countries (Campos-Mondragon et al. 2009). The groundnut seeds contain high oil percentage (50%), protein (25%), total carbohydrate (20%) and 5% fiber and ash which make a substantial contribution to human nutrition (Fageria et al. 1997). The stability and nutritional quality of groundnut oil depend on fatty acid particularly oleic and linoleic. In addition to its desirable fatty acids profile, the health benefits associated with consumption of groundnut kernel are mainly attributed to the bioactive compounds such as phenolics and total tocopherols so groundnut kernels are typically considered as a good source of antioxidant components, phytosterols with a comparatively low price (Nile and Park, 2013). Several studies have been made to produce new cultivars with improved nutritional qualities such as chemical composition, phytochemicals and high oleic/linoleic ratio (Shad et al. 2012). Recently, groundnut has been given great attention from Egyptian Government due to its importance for increasing its cultivated area in the newly reclaimed sandy soil, but these areas faces many problems such as lack of soil fertility, water limitation, increase of salinity and change of climate conditions such as high temperature.

Among non-enzymatic antioxidants, LAA is one of the most useful growth regulators to improve plant growth and yield (Shafiq et al. 2014). LAA, commonly known as vitamin C, is a water soluble and non-enzymatic antioxidant that protects cell. It acts as a co-factor in the biosynthesis of many plant hormones such as ethylene, gibberellic acid, and abscisic acid (Mazid et al. 2011). It is involved in the network of antioxidants that include ascorbate, glutathione, a-tocopherol, and a series of antioxidant enzymes (Khan and Mohammad, 2011). It acts as a substrate for ascorbate peroxidase that scavenges hydrogen peroxide. Also, it helps in the removal of superoxide radical that produced during photosynthesis and respiration processes (Smirnoff, 2011). It is involved in regulation of photosynthesis flowering and senescence (Dehghan et al. 2011; Zhang, 2013).

Therefore, the main objective of this study was to investigate the effect of foliar application of LAA as natural compound on quality and productivity of yield for three groundnut cultivars grown in sandy soil.

MATERIALS AND METHODS

Growth conditions

A pot experiments were carried out during two summer seasons 2014 and 2015 in the greenhouse of the National Research Centre, Dokki, Giza, Egypt in order to investigate the effect of foliar application at different concentrations of LAA (0, 400 and 600 ppm) on yield components, as well as some biochemical constituents in seed of tested groundnut cultivars (NC, Giza-6 and Gregory). The analysis of used soil was carried out following the methods described by Jackson, (1970) and represented in Table 1. Five seeds were sown in earthenware pots (40 cm diameter and 40 cm depth) filled by 20 kg sandy soil on June 15th during summer seasons of 2014 and 2015, respectively. Thinning was done at 30 days after planting to leave one plant per pot. Phosphorus fertilizer was added before sowing at a rate of 6.0 g per pot of calcium super phosphate (15.5% P_2O_5). Nitrogen fertilizer was applied as two equal portions at a rate of 0.60 g/pot for each in the form of ammonium nitrate (33.5%N) at 30 and 60 days after planting. Potassium fertilizer was applied as soil application at the rate of 2 g/pot in the form of potassium sulfate (48-52% K_2O) at 45 days after planting. The experiment was set up as factorial experiment in Completely Randomized Design (CRD) with three replicates.

Yield components

At harvest time (120 days after sowing), yield and yield components were determined such as number of pods/plant, pods weight/plant (g), number of seeds/plant, seed weight/plant (g), 100-seed weight (g) and oil yield/plant (g). Chemical constituents in seeds were represented by the contents of total phenols, total soluble sugars, total carbohydrates, total free amino acids and total protein, nitrogen, phosphorous, potassium, zinc, manganese, iron and oil composition were determined in the yielded seeds.

Estimation of total phenols

Total phenols were estimated in seeds dry powder according to the method described by Malik and Singh, (1980) using the Folin-Ciocalteau reagent. The absorbance was read at 650 nm and the values were expressed as μg gallic acid equivalent (GAE) g^{-1} FW.

Estimation of total carbohydrates

Total carbohydrates and total soluble sugars were estimated spectrophotometerically using the phenol-sulfuric acid method described by Dubois et al. (1956). Standard curves with glucose were prepared and the contribution of soluble sugars was calculated based on the dry weight bases.

Estimation of total free amino acids

Total free amino acids were estimated according to Sadasivam and Manickam, (1996).

Estimation of protein content

Protein content was estimated by the Bradford method using bovine serum albumin as standard (Bradford, 1976).

Estimation of mineral contents

The dried matter of the seeds digested according to the method of Chapman and Pratt, (1978). The digested solutions were stored for, nitrogen, phosphorus, potassium, zinc, manganese and iron determinations. Total nitrogen was determined by using micro-kjeldahl method (Bremner, 1996), Phosphorous was estimated using ammonium molybdate and LAA (Cooper, 1977) and potassium content by using Flame photometer, while Fe, Zn and Mn were determined by using atomic absorption apparatus (Kalra, 1998).

Estimation of oil content

The oil content of the seeds was estimated according to the procedure reported in the A.O.A.S., (1990).

Determination and identification of fatty acids

Fatty acid composition was determined quantitatively by gas liquid chromatography. The lipid samples were methylated according to Slover and Lanza, (1979). Identification of the fatty acids on the chromatogram was made by comparing the retention times of the lipid methyl esters with those of known mixtures of methyl esters run on the same column under the same conditions. The percentage of fatty acid composition was applied using the following equation:

$$\% \text{ Fatty acid} = \frac{Area\, of\; each\; peak}{Total\; area\, of\; all\; peaks}\; x\, 100$$

Statistical analysis

All obtained data of yield and its components were statistically analyzed by using MSTAT-C programme according to Snedecor and Cochran, (1980) and the combined analysis according to Steel and Torrie, (1960).

RESULTS AND DISCUSSION

Yield characteristics

Data presented in Table 2 show that exogenous application of LAA at different concentrations improved yield and its main components including number of pods/plant, pods weight/plant (g), number of seeds/plant, seeds weight/plant (g), 100-seed weight (g) and oil yield/plant (g) of groundnut cultivars (NC, Giza 6 and Gregory). The improvement depended on level of LAA and cultivar. In NC cv., application of LAA, especially at low concentration seemed to be the most effective treatment for increasing yield components. The increments reached 19.6% in pods number/plant, 55.4%, in pods weight/plant, 41.4% in seeds number/plant, 47.7 % in seeds weight/plant, 10.8% in 100-seed weight and 53.0% in oil yield/plant. On other side, in both cultivars, Giza 6 and Gregory, the most effective treatment for increasing yield components was 600 mg/l of LAA. The increments reached 68.4% and 46%, respectively in number of pods/plant, 51.7% and 74.3%, respectively in pods weight/plant; 22.5%, 72.1%, respectively in seeds number/plant, 67.7%, 51.7%, respectively in seeds weight/plant, 26.3% and 14.5%, respectively in 100-seed weight and 70.8% and 30%, respectively in oil yield/plant over the corresponding controls. Similar results were obtained by Gheeth et al. (2013) on pea, Hafez and Gharib, (2016) on wheat plants. They stated that LAA (vitamin C) has a regulatory role in promoting productivity in many plants. The beneficial effects of LAA might be attributed to its antioxidant role on enhancing protein synthesis and this may play an inductive role in improving their growth and productivity (Mazid et al. 2011). In addition, the stimulating effect of LAA on yield may be attributed to an increase in the availability and uptake of water and essential nutrients through adjusting cell osmotic pressure, and reducing the accumulation of harmful free radicals (ORS) by increasing antioxidants and enzyme activities (Hussein and Khursheed, 2014). Moreover, the increase in yield and its components might be due to positive effect of LAA on promoting plant hormones which stimulates cell division and/or cell enlargement and plant growth and this, in turn, improves yield and its components (Hassanein et al. 2009).

Total phenols content

Change in phenols content of groundnut seeds depended on the concentration of LAA and the type of cultivar (Table 3). In NC cv., foliar application of LAA treatments led to a marked decrease in total phenols content of yielded seeds as compared to control value. This decrease was more obvious with the lower concentration of LAA (400 mg/l) and represented by 29.3% compared to the control value. These results are agreed partially with those obtained by El-Hariri et al.

Table 1: Some physical and chemical properties of sandy soil.

Physical properties											
Coarse sand %		Fine sand %		Silt %		Organic matter %			Soil texture		
71.0		20.0		4.1		0.50			Sandy		
Chemical properties											
pH	EC dSm^{-1}	Cation (meq/l)			Anion (meq/l)			Available elements %			
7.7	0.11	Ca^{+2}	Mg^{+2}	Na^{+}	CO_3^{--}	HCO_3^{-}	Cl^{-}	N	P	K	
		1.68	1.20	0.98	0.21	0.98	0.56	3.9	0.44	0.28	

Table 2: Effect of foliar application of LAA on yield and yield components of some groundnut cultivars grown in sandy soil (Average of 2014 and 2015 seasons).

Yield components / Treatments		**Pods No. / plant**	**Pods weight /plant (g)**	**Number of seeds /plant**	**Seeds weight /plant (g)**	**100-seed weight (g)**	**Oil yield/plant (g)**
Cultivars	LAA mg L^{-1}						
NC	0	18.67	15.52	17.50	10.46	61.67	4.98
	400	22.33	24.11	24.75	15.45	68.34	7.62
	600	21.33	21.60	22.50	14.17	65.03	6.79
Giza 6	0	14.25	10.71	10.00	8.11	64.82	3.60
	400	21.25	16.36	13.50	10.64	77.29	4.96
	600	21.62	21.62	17.00	13.60	81.85	6.15
Gregory	0	12.50	10.23	11.00	6.98	60.00	3.97
	400	16.50	17.39	16.50	9.54	68.14	4.16
	600	18.25	17.83	18.50	10.59	68.71	5.16
LSD 0.05		2.80	3.29	4.00	2.00	8.54	1.50
Main treatments							
Cultivars	NC	20.78	20.41	21.58	13.36	65.01	6.46
	Giza 6	19.04	16.23	13.50	10.78	74.65	4.90
	Gregory	15.75	15.15	15.33	9.04	65.62	4.43
LSD 0.05		2.15	2.53	3.01	1.53	6.85	0.88
LAA	0	15.14	12.15	12.83	8.52	62.16	4.18
	400	20.03	19.29	18.25	11.88	71.26	5.58
	600	20.40	20.35	19.33	12.79	71.86	6.03
LSD 0.05		1.73	2.00	2.40	1.21	5.30	0.70

Table 3: Effect of foliar application of LAA on some chemical constituents in seeds of some groundnut cultivars grown in sandy soil (Average of 2014 and 2015 seasons).

Treatments		Chemical constituents					
Cultivars	LAA (mg/l)	Total phenols (mg/g)	Total sugars (mg/g)	Total carbohydrates (mg/g)	Total free amino acids (mg/g)	Total protein %	Oil %
NC	0	3.18	11.9	240.33	6.84	18.26	46.16
	400	2. 25	6.45	90.68	9.56	22.24	51.24
	600	2.59	7.48	150.58	8.65	19.67	48.12
Giza 6	0	1.72	7.09	170.36	7.15	16.25	45.28
	400	2.16	8.72	190.68	8.04	16.48	46.54
	600	2.89	9.10	20.95	9.13	17.01	47.39
Gregory	0	1.36	9.47	220.67	7.59	15.44	45.78
	400	2.21	10.68	240.38	8.41	17.46	46.10
	600	2.85	10.72	250.58	10.20	19.65	48.76
LSD 0.05		0.28	1.54	6.0	1.30	NS	1.53
Main treatments							
Cultivars	NC	2.67	8.61	160.53	8.35	20.06	48.51
	Giza 6	2.26	8.3	190.33	8.11	16.58	46.40
	Gregory	2.14	10.29	240.21	8.73	17.51	46.88
LSD 0.05		0.16	0.89	9.2	0.81	NS	0.88
LAA	0	2.09	9.49	210.45	7.19	16.65	45.74
	400	2.32	8.62	170.91	8.67	18.72	47.96
	600	2.66	9.10	200.70	9.33	18.78	48.09
LSD 0.05		0.15	0.86	8.6	0.66	NS	0.76

(2010) on flax plants and Shafiq et al., (2014) on canola plants. According to the results presented in Table 3, the reduction in phenolic content may be attributed to the decreasing soluble sugars which are necessary to formation of the phenolic compounds and/or due to the role of LAA in converting phenolic acids biosynthesis pathways into formation of ester organic acids (Michalak, 2006). On the contrary, total phenols content in yielded seeds of groundnut cultivars, Giza 6 and Gregory were increased in response to foliar application of LAA particularly, at high concentration. The highest increments were represented by 68.0% and 109.6%, respectively over the control values. Similar results were obtained by Sally and Mervat, (2012) on lettuce plants; El-Awadi et al. (2014) on wheat plants; Sofy et al. (2016) on *Chenopodium quinoa* plants. Accumulation of total phenol content might be due to the positive role of LAA in increment of carbohydrate biosynthesis as shown in the results of this study (Table 3). Additionally, accumulation of total phenols in response to application of LAA might be due to the stimulatory effect of ascorbate on polyphenolase enzyme activity in some groundnut cultivars.

Carbohydrate contents

Data recorded in Table 3 declare marked reductions in total soluble sugars and total carbohydrate contents in yielded seeds of NC cv. plants affected by foliar application of LAA particularly, at low concentration. The lowest values of total soluble sugars and total carbohydrates were obtained in response to low concentration of LAA represented by 6.45 and 90.68 mg g^{-1} compared with the corresponding control values (11.9 and 240.33 mg g^{-1}). The reduction in total soluble sugars and total carbohydrate contents of yielded seeds as result of LAA treatments led to conclusion that LAA increased partial utilization of carbohydrates into

Table 4: Effect of foliar application of L-ascorbate on nutrients content in seeds of some groundnut cultivars grown in sandy soil (Average of 2014 and 2015 seasons).

Treatments		Nutrients content (mg/g)					
Cultivars	LAA (mg/l)	N	P	K	Zn	Mn	Fe
NC	0	30.00	22.00	24.00	0.800	0.455	4.94
	400	36.30	27.14	31.44	0.860	0.485	5.45
	600	30.91	25.17	26.36	0.845	0.460	5.16
Giza 6	0	25.50	18.00	25.61	0.810	0.435	4.37
	400	26.41	20.01	29.23	0.820	0.450	4.53
	600	29.13	23.20	30.41	0.900	0.485	5.29
Gregory	0	26.40	20.01	28.00	0.830	0.470	6.32
	400	29.11	23.20	32.12	0.905	0.470	6.59
	600	30.03	25.14	36.45	0.925	0.615	7.63
LSD 0.05		1.90	1.46	1.49	NS	0.29	0.31
Main treatments							
Cultivars	NC	32.40	24.77	27.27	0.84	0.47	5.18
	Giza 6	27.01	20.40	28.42	0.84	0.46	4.73
	Gregory	28.51	22.78	32.19	0.89	0.52	6.85
LSD 0.05		1.70	1.30	1.12	0.05	0.25	0.25
LAA	0	27.30	20.00	25.87	0.81	0.45	5.21
	400	30.61	23.45	30.93	0.86	0.47	5.52
	600	30.02	24.50	31.07	0.89	0.52	6.03
LSD 0.05		1.25	1.16	0.62	0.05	0.23	0.24

Table 5: Effect of foliar application of LAA on fatty acids composition in seeds of some groundnut cultivars grown in sandy soil (Average of 2014 and 2015 seasons).

Fatty acids % Treatments		Saturated fatty acids			Unsaturated fatty acids					TU/TS %
Cultivars	LAA (mg/l)	Palmitic C16:0	Stearic C18:0	Total (TS)	Oleic C18:1	Linoleic C18:2	Oleic/ Linoleic	Lenolenic C18:3	Total (TU)	
NC	0	11.98	2.45	14.43	44.98	33.21	1.35	1.25	75.46	5.51
	400	11.21	2.07	13.28	49.52	29.23	1.69	1.40	83.64	6.04
	600	11.79	2.38	14.17	45.39	32.72	1.39	1.39	79.99	5.61
Giza 6	0	11.31	2.33	13.64	37.14	34.14	1.09	1.31	72.59	5.32
	400	10.52	2.33	12.85	39.01	36.80	1.06	1.45	76.69	6.01
	600	10.23	1.77	12.00	42.84	36.23	1.18	1.47	81.11	6.71
Gregory	0	12.67	2.33	15.00	39.94	38.04	1.05	1.23	78.26	5.28
	400	10.78	2.27	13.05	39.82	35.04	1.14	1.24	79.1	5.83
	600	10.60	2.24	12.84	42.77	38.67	1.11	1.41	82.85	6.45

other metabolic pathway. Interestingly, an opposite situation was observed in other cultivars, Giza 6 and Gregory. It was detected that total soluble sugars and total carbohydrate contents were incremented in yielded seeds in response to foliar application of LAA particularly, at high concentration and the increments were more pronounced in Giza 6 cv. than Gregory cv. These increments were represented by 28.35% and 20.68%, respectively in Giza 6 cv., while, the increases in Gregory cv. were 13.20%, 12.84%, respectively over the values of control plants. These results are harmony with the results of Mazher et al. (2011) on *Codiaeum variegtum* and Abdul Qados, (2014) on *Glycine max* and Sofy et al. (2016) on *Chenopodium quinoa* plants. The promotion effect of LAA on total carbohydrates in yielded seeds of groundnut plants may be due to the effective role of LAA in increasing endogenous levels of phytohormones in *Zea mays* (Hassanein et al. 2009) or by acting as an activator in photosynthesis which in turn affected total carbohydrates content (Smirnoff, 2011).

Free amino acids and protein contents

The positive effect of L-Ascorbate on total free amino acids and total protein contents in groundnut seeds in response to LAA treatments was obvious in Table 3. The stimulatory effect depended on level of LAA and cultivar. Regarding, NC cv., application of LAA, especially at low concentration seemed to be the most effective treatment for increasing total free amino acids represented by 39.77% and total protein by 21.84% compared to the corresponding untreated plants. On the other hand, the increments in the nitrogen constituents of yielded seeds of Giza 6 cv. and Gregory cv. were much more pronounced in response to 600 mg/l of LAA. The increments in total free amino acids were 27.69% and 34.39%, respectively and were 4.73 and 27.29 %, respectively for total protein content compared to control values. These results were confirmed by the earlier findings of Azooz and Al-Fredan, (2009), Hussein and Khursheed, (2014) and Sofy et al. (2016). They reported that, LAA treatments induced alterations in the enzymes related to protein metabolism, these enzymes might act as activators of protein synthesis. Moreover, the stimulation effect of LAA on protein content may be due to its role in scavenging reactive oxygen species and preventing protein oxidation and degradation (Mazid et al. 2011).

Mineral contents

The data presented in Table 4 provide evidence that foliar application of LAA increases nitrogen, phosphorus, potassium, zinc, manganese, and iron contents in yielded seeds of all groundnut cultivars compared to untreated plants but such response was depended on the concentration of LAA as well as type of cultivar. In NC cv., the magnitude response was more pronounced by applying LAA especially, at low concentration. The higher increments percentages were 21.0, 22.73, 29.17, 7.50, 6.59 and 10.33%, respectively in yielded seeds over the values of corresponding controls. However, the higher concentration of ascorbic seemed to be the more effective for increasing minerals content in yielded seeds of Giza 6 and Gregory cultivars than lower one. These increments were 14.12, 27.78, 20.00, 11.11, 11.49 and 21.10 in yielded seeds of Giza 6 cv. and 13.64, 25.00, 28.57,11.45, 30.85 and 20.74% in yielded seeds of Gregory cv. compared to untreated plants. These results could be supported by Abdul Qados, (2014) on soybean, Hussein and Khursheed, (2014) on wheat and El-Awadi et al. (2016) on lupin plants. In this connection, Talaat, (2003) mentioned that foliar application with ascorbic acid might increase root growth and the organic acids excreted from it into the soil and consequently increase the solubility of most nutrients which release slowly into the rhizophere zone where it might be utilized by the plants.

Oil content

Application of LAA had beneficial effects on oil percent of yielded seeds of groundnut plants and the responses of these plants were depended on the applied level of LAA and the cultivar type (Table 3). In NC cv., low concentration of LAA seemed to be the most effective treatment for increment oil content. This increment represented by 11% compared to the corresponding untreated plants. Regarding Giza 6 and Gregory cultivars, the increments in oil content of yielded seeds were much more pronounced in response to 600 mg/l LAA. The increments were 4.7 % and 6.5%, respectively in both cultivars compared to their control values. These results are in agreement with those obtained by Gamal El-Din, (2005) on sunflower plants; El-Awadi et al. (2016) on lupine plants.

Fatty acids composition

The results in Table 5 revealed that foliar spray of LAA at different concentrations improved oil composition by increasing unsaturated fatty acids and decreased saturated fatty acids compared to the unsprayed plants. The improvement in oil composition depended on level of LAA and tested cultivar. In NC cv., the lower concentration of LAA seemed to be more effective than the higher one in improving oil composition. Foliar application of LAA at 400 mg/l decreased palmitic and stearic acids by 6.43% and 15.51%, respectively and increased oleic, linoleic and lenolenic by 10.09%, 13.26% and 12.00%, respectively compared to their control values.

Concerning, Giza 6 and Gregory cultivars, the beneficial effect was more pronounced in response to the application of ascorbate at higher concentration than lower one, using 600 mg/L decreased the amount of palmitic and stearic acids by 9.55 and 24.03% & 16.34 and 3.86, respectively and increased Oleic (C18:1), Linoleic (C18:2) and Lenolenic (C18:3) by 15.35, 7.79 and 12.21 & 7.09, 5.40 and 14.63%, respectively. Interestingly, according to the present results, Oleic/ Linoleic and total unsaturated (TU)/total saturated (TS) were increased as a result of the application of the LAA. It could be suggested that the improvement of oil may be attributed to beneficial effects of LAA on the enzymes that catalysis the biosynthesis of the unsaturated fatty acids.

CONCLUSION

It can be concluded that, foliar application of LAA has a positive role in improving yield components, biochemical constituents and fatty acids composition in seeds of some groundnut cultivars (NC, Giza 6 and Gregory) grown in sandy soil.

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