Improvement the Growth and Quality of Green Onion (*Allium Cepa* L.) Plants by Some Bioregulators in the New Reclaimed Area at Nobaria Region, Egypt

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Abstract: The field experiments were carried out to study the effect of some bioregulators (glutathione, cysteine and methionine) on growth, quality and some biochemical constituents of green onion (*Allium cepa*, L. Giza 6 cv.) plants. Foliar spraying of glutathione, L- cysteine and L- methionine at four concentrations, 0, 25, 50, and 75mgL^{-1} after 33 days from sowing was used. Obtained results indicated that the foliar spraying of the bioregulators significantly promoted the growth and quality of green onion criteria: shoot length, white part length, bulb diameter, number of leaves, fresh and dry weight of onion plant. Leaf photosynthetic pigment contents were significantly increased by L- cysteine (25mgL^{-1}) concentration at sprout growth stage as well as L-methionine (25mgL^{-1}) concentration at vegetative growth stage. Biochemical constituents were significantly influenced by bioregulators treatments especially in plants treated with the different concentrations of glutathione, L-cysteine and L-methionine. The results cleared significant increase of the biochemical constituents; fixed oil percentage, total protein, free amino acids, phenols, flavonoids and indoles content of green onion at sprout and vegetative growth stages compared with control treatment. Total sulphur content was significantly increased by glutathione as compared with control at both growth stages of onion plant.

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1. Introduction

Onion plant (*Allium cepa* L.) is a species of the *Alinaceae* family that has a great economic importance and the second most important vegetable crop in the world (FAO, 2006). Onion plant is cultivated for ultimate uses as green and bulbs. Additionally, it has medicinal properties in the treatment and prevention a number of serious diseases (Martinez *et al.*, 2007 and Stajner *et al.*, 2008) that attributed with onion biochemical constituents.

Growth regulating substances were shown to enhance the biosynthesis of certain chemical constituents in plants. In this respect the amino acids which have a high integrity with different metabolic pools in plants were used to promote plant growth (Coruzzi and Last, 2000). PGRs also play important roles in plant adaptation to stressful environments (Huang *et al.*, 2008). Improving onion plant (*Allium cepa* L.) growth by using amino acids (methionine and cysteine) and glutathione (L-cystene, L-glutamine and L-glycine) could be through improve green onion growth physiology that reflect on build blocks of protein synthesis, which could be enzyme, hormones and antioxidants important for metabolic activities (Gilbert *et al.*, 1990).

In plants, amino acids fulfill a wide variety of functions. Their common role is to serve as building blocks of proteins, which exert manifold functions in plant metabolism, and as metabolites and precursors they are involved in plant defense, vitamin, nucleotide and hormone biosynthesis, and as precursors of a huge variety of secondary compounds. One way or the other, as active catalysts or as precursors, amino acids are essentially involved in all metabolic, regulatory, and physiological aspects of plant metabolism (Buchanan *et al.*, 2000)

Amino acid can serve as the sole source of nitrogen, which can be taken more rapidly than inorganic nitrogen (Thom *et al.*, 1980). While, exogenous amino acids decreased both ammonium influx and transporter trans-cript in root tissue (Miller *et al.*, 2007).

Sulfur is a macronutrient that is essential for plant growth and development. The most abundant form of sulfur in nature and the source of sulfur for plants is sulfate; this form is reduced and assimilated into Cys. In addition to its role as an amino acid in proteins, Cys functions as a precursor for a huge number of essential biomolecules, such as vitamins and cofactors (Wirtz and Droux, 2005), antioxidants like glutathione, which is regarded as the major determinant of cellular redox homeostasis (Meyer and Hell, 2005), and many defense compounds (Rausch and Wachter, 2005). All of these biomolecules contain sulfur moieties that act as functional groups and are derived from Cys.

In plants, cysteine biosynthesis plays a central role in fixing inorganic sulfur from the environment

and provides the only metabolic sulfide donor for the generation of methionine, glutathione, phytochelatins, iron-sulfur clusters, vitamin cofactors, and multiple secondary metabolites. O-Acetylserine sulfhydrylase (OASS) catalyzes the final step of cysteine biosynthesis, the pyridoxal 5-phosphate (PLP)-dependent conversion of O-acetylserine into cysteine. (Bonner *et al.*, 2005). Cysteine proteinases are potentially responsible for both low temperature and drought tolerance (Grudkowska and Zagdańska, 2010).

In this respect, the amino acids which have a high integrity with different metabolic pools in plants were used to promote plant growth (Coruzzi and Last, 2000). However, S-adenosyl - methionine play a role via the methyl group as a donor to produce estragole and t-anethole in cell-free extracts of the bitter fennel plant (Gross *et al*., 2002). Maxwell and Kieber (2004) indicated the link of methionine to the biosynthesis of growth regulating substances, e.g cytokinins, auxins and brassinosteroids in plants. Whereas the link of tryptophan to the biosynthesis of auxins, the phytoalexin camalexin, phenylpropanoids and other related natural products in plants was recently reported (Tao *et al*. 2008)

Low molecular weight antioxidants, such as ascorbate, glutathione, and tocopherol, are information-rich redox buffers that interact with numerous cellular components. In addition to crucial roles in defense and as enzyme cofactors, cellular antioxidants influence plant growth and development by modulating processes from mitosis and cell elongation to senescence and death (De Pinto and De Gara, 2004; Potters *et al*., 2004; Tokunaga *et al.*, 2005). In addition, the transformation and measurements of sulphur compounds within onion and garlic of putative biosynthetic intermediates have provided information about alk(en)yation of the cysteine in glutathione followed by cleavage and oxidation (Jones *et al.*, 2004). Thus, exogenous glutathione also had an inhibitory effect on growth; seedlings treatment with glutathione did not show high levels of lipid peroxidation.

Localized activity of glutathione could also help elucidate the mechanism of stress resistance. This effect indicates that glutathione may be involved in protection against DNA damage (Lodhi, 1998).

Whereas, ascorbate and glutathione are major redox metabolites in plant callus with specific roles in cellular redox homestosis and the regulation of cell cycle (Pellny *et al.*, 2009). All redox regulators influenced on the hydrolytic activity with H+ translocating enzymes, although sensitivity of proton pumps to redox regulators was found to depend from stage of plant development(Ozolina *et al.* ,2008).

Gamal El-Din and Zaki (2005) demonstrated that, plant height, number of branches per plant and dry weight of plant at full vegetative stage amino acids (Lysine, phenylalanine and L-cysteine). Many studies reported that the foliar application of amino acids caused an enhancement in plant growth, fruit yield and its components (El-Shabasi *et al*., 2005) on cucumber, (Awad *et al*., 2007) on garlic, Amin *et al* (2011) on onion and El-Awadi *el al* (2011) on snap bean.

This study aimed to clarify the effect of some bioregulators (glutathione, L-cysteine and L-methionine) on growth, quality and some biochemical constituents of green onion plants that are a one of nitrogen recovery method to nitrogen reduction and sulphure source.

2. Material and Methods

The experiments were conducted to study the effect of bioregulatores; Glutathione and amino acids (L-methionine and L-cysteine) on onion plant growth. The treatments were 0, 25, 50 and 75mgL-1) of glutathione (L-gammaglutamyl-L-cysteinylglycine), amino acids (L-cysteine HS CH_2 CH (NH_2) COOH and L- methionine CH_3 SCH_2 CH_2 CH (NH_2) COOH) and control treatment.

Onion sets (*Allium cepa* L., cv Giza 6) secured from Agriculture Research Center, Ministry of Agriculture, Cairo, Egypt. Onion sets were sown under dripe irrigation system on September 15 in two seasons 2008-2009 and 2009-2010, respectively at the experimental Station of National Research Center Nubaria region, Behira Government, Egypt. Onion sets (1cm) diameter were planted on ridges1m width and 8m length using four rows / ridge and 5cm apart. Each plot includes 2 ridges with four rows and the plot area was $16m^2$. The experiment was arranged as a complete randomized design. Onion sprouts were sprayed by the previous concentration of glutathione and amino acids (L-methionine and L-cysteine) after completely germination of onion sets (33 days after sowing). Phosphorous fertilizer as calcium super phosphate (15.5 %P_2O_5) was added pre-sowing at recommended rate 200 Kg/fed. Nitrogen fertilizer as amonium sulphate (20.5 %N) was applied at rate70Kg /fed and potassium sulphate (48% K) at rate50 Kg/fed after20days from planting.

Growth measurements

Ten plant samples were taken randomely to measure the growth criteria such as shoot length, white part length, bulb diameter, number of leaves, fresh and dry weight of plant were recorded at sprout and vegetative growth stages (48 and 63 days from planting namely A and B respectively).

Biochemical constituent's determination

Photosynthetic pigments (chl. a, b and carotenoids) were determined in fresh leaves according to **Wettstein (1957)**. Total soluble solids were determined in fresh juice using Refractometer (Shibuya-0-32, Japan). Onion plants were dried in oven at 70^{O} C and then finally ground to determine total protein **and** adsorbed nitrogen **(A.O.A.C, 1970),** free amino acid (**Plummer, 1978**), total phenols **(Daniel and Georage, 1972)** and flavonoids (**Chang *et al.*, 2002**). Fixed oil content was determined as adopted by the methods of **A.O.C.S. (1964)** with Soxhelt apparatus using petroleum ether (40-60^{o}C). Sulphur content was determined according to Dewis and Freitas (1970).

Statistical analysis

Combined analysis of the data for two growing seasons was carried out according to **Snedecor and Cochran (1980)** and the values of least significant differences (L.S.D. at 5 % level) were calculated to compare the means of different treatments.

3. Results and Discussion:

Effect of bioregulators on vegetative growth:

Data presented in Table (1) showed that the spraying onion sprouts with glutathione, L-cysteine and L- methionine treatments caused a significant increases in plant growth criteria; shoot length, white part length, bulb diameter, leaves number and fresh and dry weight of green onion plant at sprout and vegetative growth stages. Glutathione,L- cysteine and L-methionine ($25mgL^{-1}$) treatments gave the highest values of plant growth criteria at both growth stages of onion plant as compared to the other treatments and control. In this respect, $25mgL^{-1}$ of glutathione was more effective than L- cysteine and L-methinione that reflected on increasing the vegetative growth characters of green onion plant. The related increasing rates with foliar application of glutathione on shoot length, bulb diameter, fresh and dry weight per plant were 23.0, 38.4, 90.9 and 100% respectively at vegetative growth stage. The addition of an amino acids or amino acid mixture may be used to stimulate cell growth and facilitate plant regeneration (Mohamed, 1995). Many studies reported that, the foliar application of amino acids caused an enhancement effect on plant growth, fruits yield and its component on cucumber (El-Shabasi *et al*., 2005), on garlic (awadi *et al*.; 2007), on snap bean (El-Awadi *et al*., 2011) and on onion (Amin *et al* .,2011)

Glutathione is an antioxidant protecting the cell from damage caused by free radicle hydrogen. Glutathion also help the other antioxidation in the cells stay in their active form. The externally supplied glutathione similarly increased the enzyme activities, particularly peroxides (Mamdouh, 1995). In addition, it could be through improve green onion growth physiology that reflect in build blocks of protein synthesis which could be enzymes and hormones important for metabolic activates (Gilbert, *et al.*, 1990). Moreover, glutathione was more effective and defense than cysteine and methionine application. This might be due to glutathione capacity on the oxidation of free radicals in the cell. Whereas, the extremely supplied glutathione increased the enzyme activity, particularly peroxidase. Alla (1995) reported the relationship of gltutathione with corn growth and its importance for con tolerance to herbicides effects. Besides the implication of ascorbate and glutathione in the defense against oxidative stress, wheewas, these two compounds are involved in plant growth and cell cycle control (Potters *et al*., 2004).

Whereas, redoex regulators influenced on the hydrolytic activity of protein pumps to redox regulators was found to depend from stage of plant development (Ozolina *et al.*, 2008). Glutathione contents were much stronger increased in leaves of the tolerant cultivar than in the susceptible one, indicating that high levels of glutathione play an important role in the development of resistance and tolerance. The weaker increase of glutathione in the susceptible cultivar was found to be caused by low levels of glutathione precursors in glutathione producing organelles. Whereas in younger leaves of the susceptible cultivar low levels of cysteine and glutamate were found to be the limiting factor for glutathione synthesis during ZYMV-infection, low levels of glycine are limiting the availability of glutathione in this organ (Muller and Zechmann 2008).That is attributed with flux of the carbon/ amino skeleton limitis methionine and cysteine content (Hacham *et al*. 2008).This effect reflected on producing transgenic crop plants.

Effect of bioregulators on leaf pigments

Data presented in Table (2) indicated that the foliar application of glutathione, cysteine and methionine significantly increased leaf pigments (Chl. a, b and carotenoids) content at both growth stages. Cysteine ($25mgL^{-1}$) concentration was more effective than the other treatments on chlorophyll a, b and carotenoids leaf content at sprout stage as well as methionine ($25mgL^{-1}$) at the same concentration at vegetative growth stage. This reflected increasing leaf pigments content at rates 33, 22 and 20% respectively at sprout stage. These results in agreement with El-Bassiouny *et al*., (2008) mentioned that the increases in chlorophyll contents as a result of arginine and Putrescine treatments concomitantly with increasing in Mg levels in differently treated wheat plants could be attributed to the role of Mg as a structural

component of chlorophyll and reinforced the role of arginine or putrescine in chlorophyll biosynthesis. Amin *et al.* (2011) reported that the increase in photosynthetic pigment in onion leaves by the amino acid glutamine. The effect might be due to methionine and cysteine sulphur bonds that are an electron donor in the leaf which protect the cell of free radicals. In the same trend, the glutathione system also plays critical roles in the coordination of cellular processes with photosynthetic activity (Foyer and Noctor, 2009). Photosynthetic organisms have developed robust antioxidant and redox buffering systems composed of enzymatic and small molecule components (Latifi *et al*., 2009).

Glutathione is a small, ubiquitous molecule that is involved in a plethora of cellular processes in addition to its role as an antioxidant and in the maintenance of cellular redox homeostasis (Schafer and Buettner, 2001)

Effect of bioregulators on biochemical constituents

Oil percentage, total nitrogen, adsorbed nitrogen, protein, free amino acids, phenols ,flavonoids indoles and total sulphur content of green onion plant during experiment duration were significantly increased with glutathione and amino acids (cysteine and methionine) exogenous application on onion sprouts as shown in Table(3). On the other hand, total sulphur content was significantly increased compared with control treatment at both sprout and vegetative growth stages. The highest content of nitrogen, adsorbed nitrogen and free amino acids were obtained by glutathione ($50mgL^{-1}$) at vegetative stage, free amino acids by cysteine (25mgL-1) at sprout stage, nitrogen and adsorbed nitrogen by methionine $25mgL^{-1}$,oil, phenols, flavonoids and indoles content by $75mgL^{-1}$methionine concentration externally application on onion sprout or vegetative) growth stage .This effect might be due to methionine as the source of nitrogen and sulphur major nutrients and their attribution to biosynthesis of other amino acids as cysteine and glutathione as well as their protect to metabolites of the oxidation. Whereas, glutathione is crucial for biotic and abiotic stress management. It is a pivotal component of the glutathione-ascorbate cycle, a system that reduces poisonous hydrogen peroxide Noctor and Foyer (1998). It is the precursor of phytochelatins, side-chain and an antioxidant, preventing damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides **(**Pompella, *et al.* 2003).

Glutathione reduces disulfide bonds formed within cytoplasmic proteins to cysteines by serving as an electron donor. In the process, glutathione is converted to its oxidized form glutathione disulfide . Moreover, Glutathione is found almost exclusively in its reduced form, since the enzyme that reverts it from its oxidized form, glutathione reductase, is constitutively active and inducible upon oxidative stress. In fact, the ratio of reduced glutathione to oxidized glutathione (Anna *et al*., 2003) within cells is often used scientifically as a measure of cellular toxicity. Methionine is an intermediate in the biosynthesis of cysteine, carnitine, taurine, lecithin, phosphatidylcholine, and other phospholipids. Cysteine is clearly an immediate precursor of camalexin (3-thiazol-2′yl-indole), an indole phytoalexin in Arabidopsis (Glawischnig, 2007), with its component thiazole ring entirely derived from this amino acid. That based on an aldehyde acceptor species, cysteine would react directly to form a thiazolidine ring, through Schiff base formation and subsequent cyclisation via the thiol group. If GSH was to react with the aldehyde acceptor, the addition reaction would also proceed, though cyclisation to the thiazole could only occur after proteolytic processing of the GSH to yield cysteine. Intriguingly, GSH is implicated in camalexin synthesis, based on the requirement for an active γ-glutamylcysteine synthase I enzyme (Parisy *et al*., 2007). Natural substrate is pointing to a functional diversification for GSTs to include roles in isomerisation, reduction and the binding, protection and transport of secondary products.

Whether glutathione and glutathione S transferase are the immediate source of sulfur in indole glucosinolate synthesis or are indirectly involved in regulating the pathway remains to be determined. Another potential reason for the association of GSTs with glucosinolate metabolism is associated with the detoxification of breakdown products. The enzyme myrosinase breaks down glucosinolates to produce a range of products including nitriles and isothiocyanates (Fahey *et al.*, 2001), with isothiocyanates being excellent GST substrates (Kolm *et al.*, 1995).

Another group of *S*-containing natural products which are linked to GSH and the action of GSTs, though as yet lack a definitive involvement for the associated pathways are the alk(en)yl-cysteine sulfoxides , found in *Allium* species, most notably garlic and onions. Surprisingly, the synthetic route to these important *S*-containing secondary metabolites remains undefined (Jones *et al.*, 2007).In addition, moderate enhancement of glutathione can be related with GSH release from conjugates such as GSH-saccharide,porphyrinogens and other (Balounl, *et al*.,2008 and Dixon and Edwards, 2008).

Table (1): Effect of foliar spraying with bioregulators on vegetative growth criteria of green onion.

Bioregulators	g/L^{-1}	Shoot Length (cm)		White part Length (cm)		Bulb Diameter (cm)		No. of Leaves		FW (g/plant)		DW (g/plant)	
		A	B	A	B	A	B	A	B	A	B	A	B
Control	**0**	37.60	41.53	4.90	4.93	1.22	1.38	5.13	5.22	12.20	13.67	1.09	1.32
Glutathione	**25**	46.41	51.07	5.57	5.97	1.47	1.91	6.10	6.17	19.61	26.10	1.68	2.65
	50	38.12	50.78	4.80	5.70	1.22	1.54	5.40	5.53	12.16	21.79	1.09	1.93
	75	40.75	44.58	4.90	4.92	1.35	1.38	5.63	5.43	15.40	13.74	1.36	1.25
Cysteine	**25**	43.16	51.20	5.67	5.77	1.25	1.61	5.80	5.87	15.02	22.38	1.70	2.01
	50	38.69	42.71	5.40	5.43	1.23	1.59	5.33	5.80	12.07	18.39	1.07	1.85
	75	38.33	41.11	4.41	4.77	1.22	1.23	5.30	4.80	11.09	18.87	0.99	1.90
Methionine	**25**	41.49	48.68	5.33	5.50	1.33	1.50	5.70	5.40	15.76	20.44	1.27	1.86
	50	38.99	41.08	5.07	5.12	1.27	1.44	5.50	5.28	11.22	13.60	0.98	1.34
	75	39.53	41.33	5.03	5.06	1.27	1.46	5.53	5.27	12.91	14.75	1.15	1.46
L.S.D 5%		2.85	2.71	0.50	0.53	0.10	0.16	0.61	0.78	2.50	2.64	0.61	0.32

A: Sprout stage B: Vegetative stage

Table2: Effect of foliar spraying with bioregulators on photosynthetic pigments in green onion leaves (mg/g-1)

Bioregulators	mgL^{-1}	Chla		Chlb		Cart	
		A	B	A	B	A	B
Control	**0**	0.288	0.249	0.152	0.101	0.177	0.171
Glutathione	**25**	0.310	0.268	0.155	0.114	0.190	0.159
	50	0.297	0.222	0.142	0.098	0.192	0.158
	75	0.262	0.266	0.140	0.076	0.163	0.125
Cysteine	**25**	0.383	0.287	0.162	0.110	0.216	0.149
	50	0.292	0.264	0.137	0.107	0.177	0.137
	75	0.303	0.291	0.143	0.102	0.163	0.129
Methionine	**25**	0.291	0.295	0.145	0.115	0.213	0.181
	50	0.274	0.286	0.143	0.110	0.186	0.160
	75	0.270	0.262	0.133	0.095	0.177	0.151
LSD 5%		0.015	0.018	0.007	0.012	0.013	0.036

A: Sprout stage B: Vegetative stage

Table 3: Effect of bioregulators on biochemical constituents of green onion

Bioregulators	mgL^{-1}	Oil %		Total N (%)		Absorbed N/plant		Total Protein (%)		Free Amino Acids(mg/g)		Phenols (mg/g)		Flavonoids (mg/g)		Indoles (mg/g)		Sulpher Content (mg/l)	
		A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Control	**0**	5.49	5.54	1.25	2.27	1.36	2.99	7.81	14.17	48.40	28.06	2.84	9.48	2.61	5.80	7.87	19.50	1.53	1.47
Glutahthione	**25**	6.26	5.62	1.38	2. 87	2.32	4.98	8.63	16.15	47.14	54.79	3.78	8.69	2.61	4.95	7.95	15.86	1.31	0.94
	50	6.18	6.08	1.43	2.58	1.57	7.58	8.96	17.92	42.84	56.52	4.07	8.27	2.59	4.35	9.37	17.23	2.43	2.20
	75	6.41	6.09	1.69	2.40	2.29	3.00	10.54	15.00	41.96	40.33	5.73	9.26	2.68	4.99	10.40	18.52	1.76	1.90
Cysteine	**25**	9.66	5.81	1.53	2.62	1.79	5.24	9.58	16.35	53.10	30.71	4.30	9.16	2.41	5.29	7.69	20.23	1.42	1.43
	50	9.39	5.81	1.45	2.30	1.56	4.27	9.06	14.38	42.47	31.81	5.25	7.79	2.60	5.34	8.67	19.87	1.21	1.99
	75	7.87	6.33	1.50	2.33	1.48	2.10	9.38	14.58	42.78	35.10	5.27	9.74	2.60	5.40	9.55	19.50	1.43	1.50
Methionine	**25**	9.71	6.13	3.26	2.10	4.13	3.90	20.38	13.13	48.56	27.24	5.12	9.77	2.59	5.60	8.67	17.18	1.33	1.33
	50	9.88	6.33	2.90	1.97	2.84	2.63	18.13	12.29	44.60	25.51	4.67	7.55	2.39	4.86	9.30	19.80	1.07	1.06
	75	9.88	6.60	2.70	2.46	3.10	3.59	16.88	15.38	51.38	30.21	4.87	9.80	2.76	5.47	10.18	20.41	1.56	2.30
LSD 5%		0.22	0.38	0.18	0.22	0.33	0.61	1.15	1.38	1.23	2.08	0.29	0.40	0.06	0.23	0.61	0.78	0.089	0.084

A: Sprout stage B:Vegetative stage

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