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RESPONSE OF GROWTH, YIELD AND YIELD QUALITY OF WHEAT (*Triticum aestivum* L.) TO ASCORBIC ACID OR NICOTINAMIDE APPLICATION IN NEWLY RECLAIMED SOIL

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ABSTRACT

Two field experiments were carried out during two successive seasons of 2005/06 and 2006/07 at the Agricultural Production and Research Station, National Research Centre, El Nubaria Province, El Behaira Governorate, Egypt. The experiments aimed to study the effect of ascorbic acid or nicotinamide as soaking grains with 10 and 20 mg/l or as foliar spraying with ascorbic acid at 100 and 200 mg/l or nicotinamide at 25 and 50 mg/l on wheat plants. The results indicated that, different treatments of ascorbic acid or nicotinamide induced increments regarding plant height, dry weight, relative water content (RWC) as well as photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) compared with control plants. Moreover, the applied treatments increased yield and yield component traits (spike length, spike weight, spikelets and grains number/spike, grains weight/spike, spikes number/m^2, 1000-grains weight as well as grains; straw and biological yield (ton/fed). Also, the results showed increases in polysaccharide, total carbohydrates, total soluble-N and crude protein contents of the yielded grains. Moreover, application of ascorbic acid or nicotinamide increased amino acid content compared with control plants. The predominant amino acids were glutamic acid and proline. This study reveals that pre-soaking or spraying of wheat plants with ascorbic acid or nicotinamide could effectively enhance growth and increase productivity of plant as well as improving the chemical constituents of the yielded grains.

Keywords: Ascorbic acid - nicotinamide - wheat - growth - yield -chemical composition – sandy soil.

INTRODUCTION

Wheat is one of the important cereal crops in Egypt as well as many other countries in the third world. There is an extreme shortage in wheat production in Egypt, such problem appears to be growing rather than diminishing or even stabilizing due to the increase of population rate and limited cultivated area. This could be achieved by producing more productive cultivars and/or sowing wheat plant in the newly reclaimed area.

Some regions of the world such as parts of the Sahara Desert of Northern Africa " Arid and semi-arid zones" are defined as areas in which plants are exposed to a combination of environmental stress conditions, including low water availability, high irradiance, temperature fluctuations and nutrient deprivation. These regions receive an average of 5 mm of rainfall or less per year. Nonetheless, most of the world's agriculture is subjected to low field capacity. Such stresses may lead to an imbalance between antioxidants defense and the amount of activated oxygen species (AOS) resulting in oxidative stress. The water stress may be alleviated by irrigation whenever possible or by using certain antioxidants (Asada, 1999).

Vitamines could be considered as bioregulators which in relatively low concentrations exerted a pronounced influence on plant growth and development (El- Bassiouny *et al.*, 2005). Ascorbic acid and nicotinamide are known as growth regulating factors that influence many physiological processes such as synthesis of enzymes, nucleic acids and protein as well as acting as coenzymes (Tarraf, 1999).

Ascorbic acid (vitamin C) is a water soluble antioxidant molecule which protects plant cells (Blokhina *et al.*, 2003) and acts as primary substrate in the cyclic pathway for enzymatic detoxification of hydrogen peroxide (Shalata and Neumann, 2001).

Nicotinamide is a well characterized constituent of the pyridine dinucleotide coenzymes NADH and NADPH which are involved in many enzymatic oxidation reduction reactions in living cells (Berglund, 1994). Nicotinamide improves the induction of defensive metabolism involving secondary metabolite biosynthesis and/or the manifestation of defense metabolism in plants (Berglund and Ohlsson, 1995). Moreover, nicotinamide improved plant growth and yield of wheat (Foda, 1987) and broad bean (Radi *et al.*, 2001).

This work is an attempt to improve the growth, yield and yield quality of wheat plants grown under newly reclaimed sandy soil by using of ascorbic acid or nicotinamide.

MATERIALS AND METHODS

Two field experiments were carried out at the Agricultural Production and Research Station, National Research Centre, El Nubaria Province, El Behaira Governorate, Egypt during two successive years of 2005/06 and 2006/07. The soil texture of the experimental site was sandy soil and having the following characters: sand 91.20%, silt 3.70%, clay 5.10%, pH 7.40%, $CaCO_3$, 1.40%, EC 0.50 mmllos/cm^3 and the available total N, P and K were 8.10, 3.20 and 20.0 ppm, respectively at 0-30 cm depth according to the method described by Chapman and Pratt (1978).

The soil was ploughed twice and divided into plots, during seed preparation, 150 kg calcium superphosphate/fed (15.5% P_2O_5) was used as a general application.

The experiment includes the following treatments:

T1. Control 1 (soaking grains for 6 hours in water)
T2. Soaking grains for 6 hours in 10 mg ascorbic acid /l.
T3. Soaking grains for 6 hours in 20 mg ascorbic acid /l.
T4. Soaking grains for 6 hours in 10 mg nicotinamide/l.
T5. Soaking grains for 6 hours in 20 mg nicotinamide/l.
T6. Control 2 (spraying plants with water at 45 and 60 days after sowing (DAS).
T7. Spraying plants with 100 mg ascorbic acid /l at 45 and 60 DAS.

T8. Spraying plants with 200 mg ascorbic acid/l at 45 and 60 DAS.
T9. Spraying plants with 25 mg nicotinamide/l at 45 and 60 DAS.
T10. Spraying plants with 50 mg nicotinamide/l at 45 and 60 DAS.

The treatments were arranged in Completely Randomized Block Design (CRBD) with four replicates. The experimental unit consisted of 20 rows 15 cm apart and 3.50 meters along (10.50 m^2). Wheat grains of cultivar Sakha-93 sown by drilling seed manually in the rows at 15-cm apart at the rate of 60 kg/fed (fed=4200 m^2). Sowing date in both seasons was around mid of November. 50 kg potassium sulphates (48% K_2O)/fed was added after 30 days from sowing. 120 kg N/fed as ammonium sulphate (20.6% N) was added in six equal doses after complete germination and every two weeks till begining spike emergences stage. Sprinkler irrigation was applied as plants needed. Wheat plants were manually harvested on the first week of May in both seasons.

Growth parameters:

During both growing seasons, wheat plants from each plot -in one square meter- were cut from ground surface at 75 days after sowing. Shoot height and dry weight of shoot/m^2 were determined. Dry weight was determined after drying in a forced oven at 70˚C till constant weight.

Photosynthetic pigment and relative water content:

Photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) in fresh leaves were determined spectrophotometrically as method recommended by Moran (1982). Relative water content (RWC) was determined according to Henson *et al.* (1981) using the following formula:

RWC = 100×(fresh mass – dry mass)/(Turged mass – dry mass)

Turged mass was determined after saturation of leaf blades in distilled water sealed glass tube for 4 hours at room temperature followed by overnight storage at 5˚c. The dry mass was determined after 48 hours at 80˚c.

Yield and yield components:

One square meter from each plot was counted to determine number of spikes/m^2. Plant height, number of spikelets and grains per spike, length and weight of spike, grains weight of spike and 1000–grains weight were determined from randomly selected 20 tillers from each plot. Wheat was threshed manually to determine grains, straw and biological yield per plot (3 × 3.5m) that was converted into ton per fed.

Chemical composition of grains:

The dried grains were finally ground. Total soluble carbohydrates and sucrose were determined using modifications of the procedures of Yemm and Willis, (1954) and Handel (1968). Total carbohydrate content was determined colorimetrically according to Dubois *et al*.,(1956). Polysaccharides were calculated by the difference between total carbohydrates and total soluble carbohydrates. Total soluble–N and protein contents were determined by the Kjeldahl method of Pirie (1955). Hydrolysis of amino acid of wheat grains was carried out according to Gehrke *et al*. (1985) and performed on Eppdrof-Germany LC 3000 amino acid analyzer.

Statistical analysis:

The data recorded were subjected to the statistical analysis by M-STAT-C statistical analysis program (MSTAT, 1988). Since the trend was similar in both seasons, Bartlett's test (1937) and the combined analysis of the two growing seasons were done. Least significant difference test was applied at 0.05 probability level to compare mean treatments (Snendecor and Cochran, 1989).

RESULTS AND DISICUSSION

Growth parameters and relative water content:

The growth response of wheat plants to different treatments of ascorbic acid or nicotinamide are represented in (Fig. 1). Data show that, soaking grains with ascorbic acid or nicotinamide (10 and 20 mg/l) as well as foliar treatment with ascorbic acid (100 and 200 mg/l) or nicotinamide (25 and 50 mg/l) had stimulatory effect on plant height and dry weight of wheat plants (g/m^2) in comparison with the untreated plant. The magnitude of these increments was observed with the increase in concentrations of either ascorbic acid or nicotinamide. Soaking treatments with ascorbic acid was more effective than spraying treatment while the reverse was true for nicotinamide. Maximum significant increase in growth parameters resulted from spraying treatment with 50 mg/l nicotinamide.

Relative water contents (RWC) of wheat plants increased in response to ascorbic acid or nicotinamide either by soaking or spraying treatments over the control. In general, soaking treatments were more effective than foliar spraying.

The increase in growth parameters in response to ascorbic acid treatments could be attributed to the effect of ascorbic acid on many biochemical processes such as activating the SH-enzymes (Mapson,1958) as well as its stimulatory effect on photosynthesis and increasing carbohydrate contents (Dhopte and Lall, 1987). The role of ascorbic acid in plant growth was reported by Smirnoff (1996) and Blokhina *et al.,* (2003) which involved as antioxidant defense, photoprotection and regulation of photosynthesis and growth. These results are in agreement with those reported by Arisha, (2000); Youssef and Talaat (2003) and Gamal El-Din (2005) on different plant species. With regard to the effect of nicotinamide, El–Bassiouny *et al.*, (2005) reported that the increase in the growth and development of faba bean plants in response to nicotinamide treatments might be due to the enhancement of cell division and/or cell enlargement and/or influence on DNA replication. Also, our results are in agreement with those of El-Lithy and El-Greadly (2001) on melon plant; Balbaa and Refaat (2002) on *Tagetes minuta* and El-Bassiouny (2005) on wheat plant.

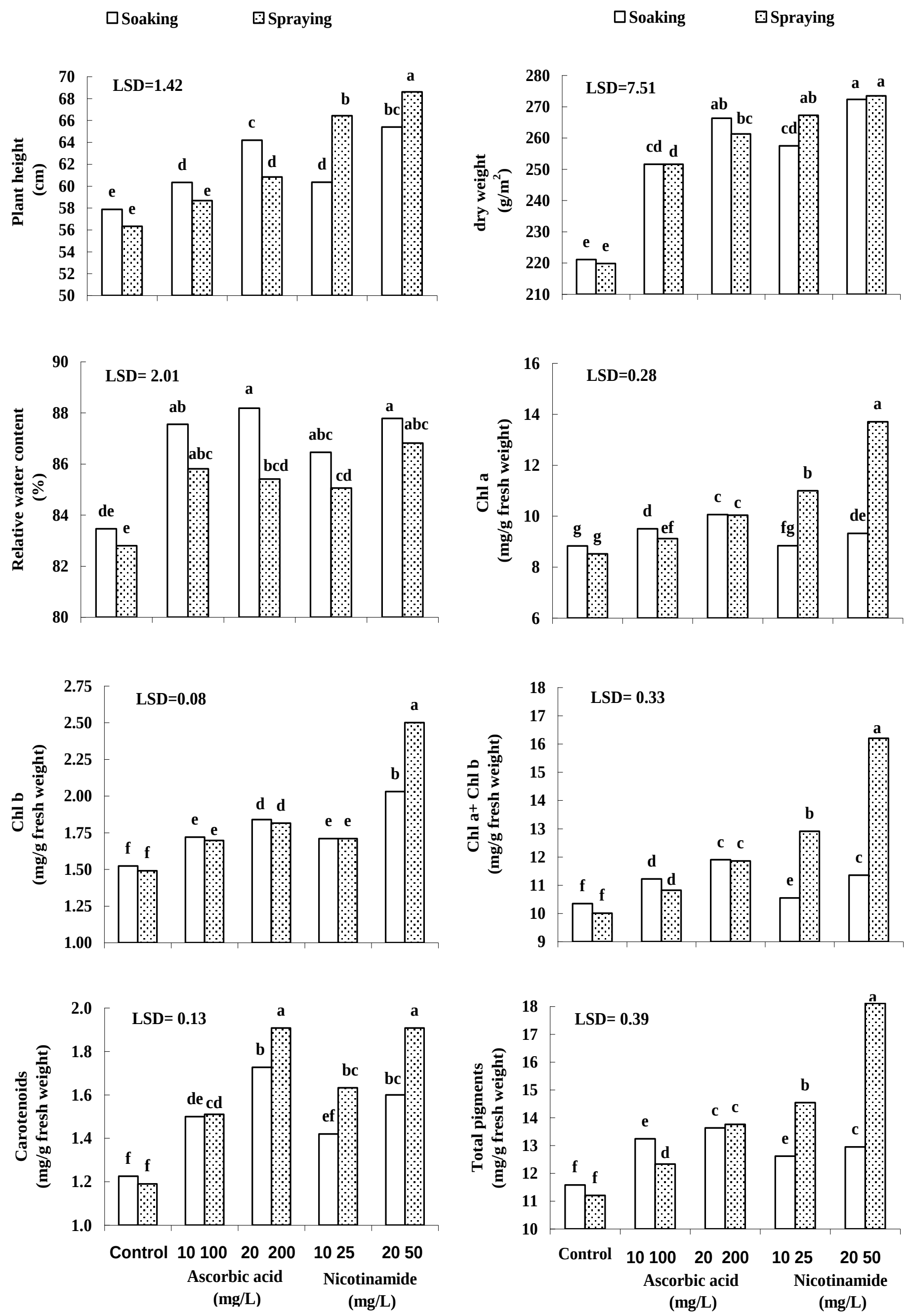

Fig. (1): Effect of ascorbic acid or nicotinamide on growth parameters and photosynthetic pigments of wheat at 75 days from sowing. (combined data over two seasons).

Vertical bars within each Fig. having the same letter(s) are not significantly different at the 0.05 level of probability Least Significant Difference Test (LSD)

Photosynthetic pigments:

Application of ascorbic acid or nicotinamide on wheat plants, either by soaking grains or foliar treatment significantly increased chlorophyll a, chlorophyll b, carotenoids contents, total chlorophylls (a + b) and total pigments in most cases (Fig. 1). The magnitude of increase is much more pronounced by applying foliar treatment with 50 mg/ l nicotinamide. Our results in response to ascorbic acid agree with those reported by El-Ghamriny *et al*., (1999) on tomato plants, and El–Bassiouny *et al*., (2005) on faba bean plant. In addition, Ghourab and Wahdan (2000) indicated that ascorbic acid increased photosynthetic efficiency, leaf area and delay senescence of cotton plants. Similar results have been reported in response to nicotinamide on wheat plant (Foda, 1987). The effect of nicotinamide on the biosynthesis of chlorophyll may be attributed to its activation of enzymes that regulate photosynthesis as mentioned by (Taylor *et al*., 1982). In addition to, the role of ascorbic acid or nicotinamide in protecting chloroplast from oxidative damage (Munne–Bosch *et al.* 2001).

Yield and yield components:

The results in Table (1) indicate that soaking wheat grains or foliar spraying with ascorbic acid or nicotinamide improved plant height and yield component traits i.e., spike length and weight, number of spikelets and grains/spike, grains weight/spike, number of spikes/m^2 and 1000-grains weight. Moreover, all treatments caused significant increases in grains; straw as well as biological yield (ton/fed) (Table 2). In general, it appears that soaking treatments with ascorbic acid are more effective than foliar treatments and vice versa in case of nicotinamide. Foliar spraying with nicotinamide at 50 mg/l proved to be more effective in increasing yield and yield components. Generally, different treatments increased grain yield by 11.34-22.16 %, straw yield by 9.18-20.75 % and biological yield by 10.65-15.16%, respectively over the untreated plant.

The increase in grains weight might be ascribed to the promotive effect of ascorbic acid or nicotinamide in increasing the assimilates and their translocations from leaves to the spike where grains weight increased. Also, the increase in the grain yield of wheat by application of ascorbic acid or nicotinamide could be a result from increasing the number of spikes/m^2 and improving the others yield components (Table1). In respect to the effect of ascorbic acid, our results are in agreement with those reported by of El-Ghamriny *et al*. (1999) on tomato and Arisha (2000) on potato. Givan (1979) reported that ascorbic acid is a product of D-glucose metabolism which affects nutritional cycles activity in higher plants and plays an important role in the electron transport system. El-Bassiouny (2005) emphasized the positive effect of nicotinamide on wheat. Moreover, El-Bassiouny, *et al* (2005) indicated that the increase in yield and yield components of faba bean could be attributed to the effect of ascorbic acid or nicotinamide on enhancing protein synthesis and delaying senescence.

T1

Chemical composition of grains:

The levels of sucrose, polysaccharides and total carbohydrates increased significantly due to ascorbic acid or nicotinamide application as compared to the control treatment (Fig. 2). However, total soluble carbohydrates decreased in response to all treatments except in case of soaking treatments with ascorbic acid which showed an increase. The maximum increase in total carbohydrate contents (25.91%) and sucrose (64.77%) resulted from soaking treatment with 20mg/l ascorbic acid. Meanwhile, the maximum increase in polysaccharide content resulted from foliar treatment with 50mg/l nicotinamide. The highest decrease in total soluble carbohydrates resulted from foliar treatment with 100 and 200 mg/l ascorbic acid.

The increase in carbohydrate content in the yielded wheat grains resulted from the different treatments could be attributed to the high contents of photosynthetic pigments (Fig. 1) and through the effect of ascorbic acid or nicotinamide on enhancing carbohydrate synthesis and/or decreasing its breakdown. Similar results were obtained on different plant species El-Shazly and El-Masri (2003); Reda and Gamal El-Din (2005) and El-Bassiouny (2005).

The different treatments of ascorbic acid or nicotinamide effectively increased total soluble–N and total protein (%) of the wheat grains compared with those of the untreated plants.

Spraying treatment with 50 mg/l nicotinamide caused the highest significant increment in protein content (15.93%) over the control. In this respect, Balbaa and Refaat (2002) and El-Bassiouny *et al.* (2005) reported that nicotinamide increased nitrogen contents of *Tagetes minuta* and faba bean plants, respectively.

Table (2): Effect of ascorbic acid or nicotinamide on yield of wheat (ton/fed.) and HI (%). (combined data over two seasons)

Charcters / Treatments	Yield (ton/fed.)			Harvest index (%)
	grains	Straw	Biological	
*T1. Control 1(soaking grains in water)	1.94 [f]	2.94 [f]	4.88 [d]	39.75 [b]
T2. Soaking grains in 10 mg ascorbic acid/l	2.21 [d]	3.28 [cde]	5.49 [c]	40.23 [ab]
T3. Soaking grains in 20 mg ascorbic acid/l	2.31 [b]	3.40 [a-d]	5.71 [b]	40.46 [ab]
T4. Soaking grains in 10 mg nicotinamide/l	2.16 [e]	3.24 [de]	5.40 [c]	40.03 [ab]
T5. Soaking grains in 20 mg nicotinamide/l	2.32 [ab]	3.48 [ab]	5.80 [ab]	39.98 [ab]
**T6. Control 2 (spraying plants with water)	1.93 [f]	2.96 [f]	4.89 [d]	39.47 [b]
T7. Spraying plants with 100 mg ascorbic acid/l	2.27 [c]	3.21 [e]	5.48 [c]	41.42 [a]
T8. Spraying plants with 200 mg ascorbic acid/l	2.34 [ab]	3.33 [b-e]	5.67 [b]	41.26 [a]
T9. Spraying plants with 25 mg nicotinamide/l	2.31 [b]	3.44 [abc]	5.75 [ab]	40.17 [ab]
T10. Spraying plants with 50 mg nicotinamide/l	2.36 [a]	3.55 [a]	5.91 [a]	39.91 [ab]
LSD at 5%	0.04	0.17	0.17	ns

*T1 - T5: Grains soaked for 6 hours before sowing

**T6 - T10: Plants sprayed at 45 and 60 days from sowing.

Means within a column, followed by the same letter (s) are not significantly different at 5% level of probability by Least Significant Differences Test (LSD).

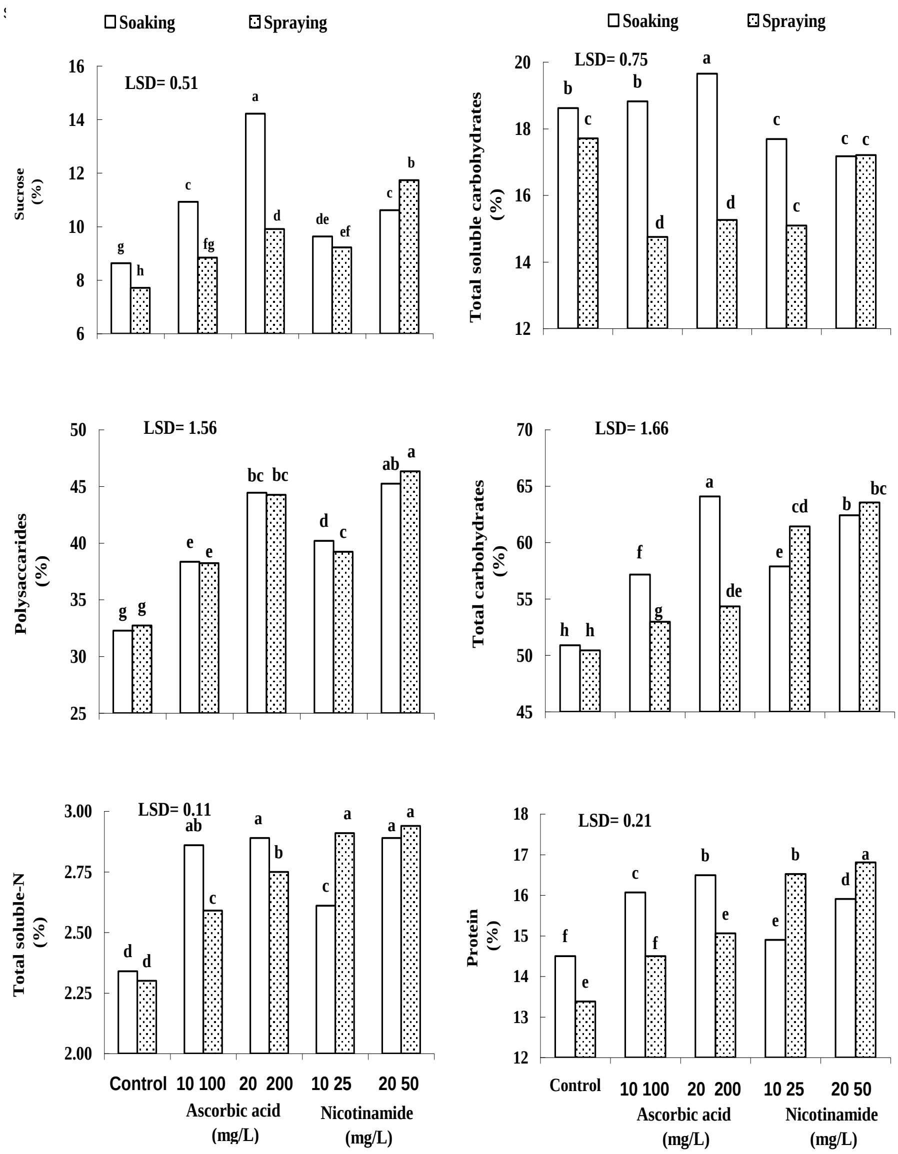

Fig. (2): Effect of ascorbic acid or nicotinamide on grains quality of wheat (combined data over two seasons).

Vertical bars within each Fig. having the same letter(s) are not significantly different at 5% level of probability by Least Significant Difference Test.

Amino acid contents:

Soaking wheat grains or spraying wheat plants with the different concentrations of ascorbic acid or nicotinamide increased the total amino acid contents as compared with the untreated plant (Table 3). Soaking wheat grains with 20 mg/l ascorbic acid and spraying wheat plant with 50 mg/l nicotinamide were the most effective treatments. The increase in total amino acids reached 35.35% and 34.41% in case of ascorbic acid and nicotinamide, respectively.

Table (3): Effect of ascorbic acid or nicotinamide on amino acid composition (mg/100g dry weight.) of grains wheat.

Treatment / Amino acides	Soaking					Spraying				
	Control 1	Ascorbic acid (mg/l)		Nicotinamide (mg/l)		Control 2	Ascorbic acid (mg/l)		Nicotinamide (mg/l)	
		10	20	10	20		100	200	25	50
Aspartic	16.34	24.87	26.39	24.98	18.96	12.21	10.14	21.04	13.01	25.17
*Thereonine	5.66	10.91	10.29	9.41	6.96	4.10	4.32	10.13	4.59	11.38
Serine	12.93	18.35	16.80	14.14	12.71	9.58	8.24	19.25	8.91	21.65
Glycine	8.73	13.70	14.92	13.87	10.65	6.79	6.67	13.49	8.87	13.48
Alanine	7.18	10.62	13.73	12.24	10.20	5.76	5.29	11.14	7.29	11.38
Cysteine	20.52	29.77	36.85	31.60	35.00	12.77	18.78	30.54	23.97	49.34
*Methionine	0.72	0.00	0.15	0.19	0.16	0.00	0.00	0.00	0.00	0.00
*Isoleucine	7.21	9.85	9.73	8.53	9.23	4.47	5.36	9.87	6.28	9.30
*Leucine	11.72	14.31	20.23	16.87	17.65	7.96	10.26	11.22	12.08	18.16
*Tyrosine	4.84	5.85	6.64	4.67	5.85	2.38	2.89	5.76	3.81	6.05
*Phenylalanine	7.17	9.51	13.86	11.21	10.17	5.57	7.11	12.04	7.39	17.54
Histidine	5.43	6.80	6.08	4.69	4.31	3.57	5.41	7.56	3.25	6.28
*Lysine	5.37	6.78	7.91	4.76	5.41	2.77	2.46	7.55	3.65	7.18
Arginine	6.11	9.91	10.94	5.11	6.40	3.46	3.42	6.36	3.78	10.46
Glutamic	115.02	128.14	131.17	120.00	115.94	86.82	85.88	136.79	97.10	107.54
*Valine	0.48	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00
Proline	169.55	190.65	227.85	237.54	217.34	129.29	160.52	179.08	163.28	187.14
Total amino cids	408.98	490.02	553.14	518.81	486.94	298.93	360.82	481.95	367.26	502.05
* Essential	**71.39**	**97.84**	**116.04**	**91.37**	**86.29**	**44.67**	**57.12**	**95.40**	**64.99**	**129.64**
Non- essential	**337.59**	**392.18**	**437.50**	**427.44**	**400.65**	**254.26**	**303.70**	**386.55**	**302.27**	**372.41**
Ess./non-ess.	**0.211**	**0.290**	**0.265**	**0.214**	**0.227**	**0.177**	**0.181**	**0.247**	**0.215**	**0.348**

Also, the data show that all treatments increased the essential amino acids such as threonine, histidine, metheonine, isoleucine, leucine, phenylalanine and lysine. In addition, the ratio of total essential to non essential amino acids was increased especially with spraying of 50 mg/l nicotinamide treatment. With regard to proline content, which is the most important organic solute for the defense against drought (Pustovoifova *et al*., 2000). Also, the data show high increments in proline due to the different treatments of ascorbic acid or nicotinamide. In this respect, Al-Hakimi and Hamada (2001) found that soaking wheat grains in ascorbic acid induced accumulation of proline.

It could be concluded from this study that wheat pre-soaking or spraying of wheat plants with ascorbic acid and nicotinamide could effectively enhance growth and increase productivity of wheat as well as improving the chemical constituents.

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استجابة نمو و محصول وجودة نبات القمح لحمض الأسكوربيك أ والنيكوتين أميد فى الأراضي المستصلحة حديثا

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أجريت تجربتين حقليتين خلال المواسم الشتوية ٢٠٠٥/٢٠٠٦ و ٢٠٠٦/٢٠٠٧ في مزرعة المركز القومي للبحوث – النوبارية – محافظة البحيرة بهدف دراسة تأثير كل من حمض الأسكوربيك أو النيكوتين أميد سواء نقع البذور بتركيزات ١٠ و ٢٠ ملليجرام/لتر أو الرش بتركيزات ١٠٠ و ٢٠٠ملليجرام/لتر لحمض الأسكوربيك و ٢٥ و٥٠ ملليجرام/لتر للنيكوتين أميد على نبات القمح. أدت المعاملات المختلفة إلي زيادة في طول النبات والوزن الجاف والمحتوى المائي بالإضافة إلي صبغات البناء الضوئي مقارنة بالنباتات غير المعاملة وذلك بعد ٧٥ يوم من الزراعة. كما أدى نقع البذور أو رش النباتات عند ٤٥ و ٦٠ يوم من الزارعة بحمض الأسكوربيك أو النيكوتين أميد بالتركيزات المختلفة إلى زيادة المحصول كما ونوعا من حيث (طول السنبلة , وزن السنبلة , عدد السنيبلات والحبوب لكل سنبلة , وزن الحبوب/ سنبلة , عدد السنابل / متر مربع ووزن ١٠٠٠ حبة . وكذلك محصول الحبوب / فدان , القش / فدان وكذلك المحصول البيولوجي).هذا بالإضافة إلي أن كل المعاملات آدت إلى زيادة في نسبة الكربوهيدرات وزيادة نسبة البروتين الكلى في البذور الناتجة مقارنة بالنباتات الغير معاملة. كما أدى أستخدم كل من حمض الأسكوربيك والنيكوتين أميد إلي زيادة في المحتوى من الأحماض الأمينية وخاصة الأحماض الأمينية الأساسية وزيادة كبيرة فى البر ولين وحمض الجلوتاميك مقارنة بالنباتات غير المعاملة.

يستنتج من هذه الدراسة ان نقع حبوب القمح قبل الزراعة أو رش النباتات بعد ٤٥ و ٦٠ يوم من الزراعة بحمض الأسكوربيك أو النيكوتين أميد بالتركيزات المختلفة يؤدى الى تحسين نمو النباتات و يزيد من انتاجية القمح كما و نوعا فى الاراضى المستصلحة حديثا.