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Physiological Responses of Wheat Plant to Foliar Treatments with Arginine or Putrescine

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Abstract: Two pot experiments were carried out in the screen of National Research Centre during two successive seasons 15/11/2003/04 - 2004/05 to study the effects of foliar application of arginine or putrescine (0.0, 0.6, 1.25, 2.5 and 5 mM) at three physiological stages (vegetative, 30 DAS; just before emergence of main spike, 60 DAS and during grain filling, 90 DAS) on different growth criteria, yield components, endogenous bioregulators, and biochemical changes. The data were taken at 75 days after sowing (DAS) and the yield at 135 DAS. All treatments significantly reduced wheat plant height. In the meantime, all growth parameters were significantly increased in response to arginine and putrescine applications at 30 or 60 DAS. Moreover, the same treatments increased significantly yield components of treated wheat plants at 30, 60 or 90 DAS more than those of untreated ones. The applied treatments on wheat plants at 30 or 60 DAS increased significantly the endogenous phytohormones; auxins (IAA), gibberellins (GAs) and cytokinins, meanwhile, abscisic acid (ABA) was decreased as compared with the untreated plants. The applied treatments significantly increased the content of photosynthetic pigments, carbohydrate, nucleic acids (DNA and RNA) and nitrogenous constituents over the control values. Also the applied treatments significantly increased the mineral contents (K, Ca, Mg and P) of wheat shoots in comparison to the untreated plants. Protein electrophoratic pattern of the treated wheat plants at 75 day old revealed that, arginine or putrescine application at 30 or 60 DAS induced the appearance of 5 new protein bands at molecular weights 222, 131, 93, 50 and 14 KDa. Spraying plants at the vegetative stage (30 DAS) with 2.5 mM of either arginine or putrescine could be considered the most suitable conditions induced the highest growth parameters, physiological characters, endogenous phytohormones of wheat plants and consequently enhanced the yield quantity and quality.

Key words: Arginine, Putrescine, Wheat, Endogenous phytohormones, Photosynthetic pigments, Carbohydrate, DNA, RNA, Nitrogenous constituents, Protein electrophoretic pattern, mineral contents.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is considered one of the most widely grown crops of high nutritive value in the world as well as in Egypt. The wheat grains contain large amounts of proteins, carbohydrates in addition to some mineral and vitamins. In Egypt, wheat has a special importance because the local production is not sufficient to meet the annual demands. In Egypt, wheat sown at normal sowing date (1-15) November may be exposed to high temperature stress during grain filling (at March or April) due to the hot wind of El-Khamaseen for one or more days which in turn reduces growth, yield and quality of grains mainly by shortening the reproductive and ripening growth phases (Nagarajan, 2002; Singh and Pal, 2003). Therefore, increasing the local production of wheat is the target to cover the local consumption. This could be achieved by introducing more productive varieties, improving the culture practices such as sowing the wheat in the newly reclaimed area or application of some growth promoters during different growth stage.

Polyamines are low molecular mass polycation compounds found in all living organisms. They are essential cell components and growth regulators in plants as well as other living organisms (Glaston and Kaur – Sawhney, 1995). hey play a critical role in different biological processes, including cell division, growth, somatic embryogenesis, floral initiation, development of flowers and fruits (Davies, 1995). Also, they are effective retardant of senescence (Couee *et al*., 2004 and Tang *et al*., 2004).

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The amino acids arginine and ornithine are considered the main precursors of polyamines mainly putrescine (Put) (Pandy, *et al.,* 2000). Via putrescine and methionine the other PAs are synthesized. So, some physiologists use arginine as a precursor of putrescine (Nassar *et al.,* 2003).

Moreover, Paschalidis and Roubelakis–Angelakis (2005) reported that, polyamines, their precursor arginine and their biosynthetic enzymes are involved in the stimulation of cell division, expansion & differentiation and vascular development in tobacco plant. Pritsa and Voyiatzis (2004) found a direct relationship in terms of time of polyamine fluctuations with developmental processes such as floral differentiation, anthesis fertilization and fruit development in olive. Nassar *et al.* (2003) proved that addition of arginine or putrescine induced significant increases in growth (fresh and dry weights) of bean plants. Gupta *et al.* (2003) proved also that, putrescine (10^{-5}M) increased grain yield, biological yield and seed weight index of wheat plant.

.Putrescine and spermidine application provoked increase in indole acetic acid (IAA) level to limited extent of wheat shoots (Iqbal *et al.,* 2006). Also, El-Bassiouny (2004) and Bekheta and El-Bassiouny (2005) illustrated that; exogenous application of putrescine on *Pisum sativum* and wheat, respectively, increased the amount of endogenous promoters (indole acetic acid, gibberellins and cytokinins) and decreased abscisic acid.

Polyamines play an important role in the photosynthetic system. Their potential role was illustrated in the studies of HuiGuio *et al.* (2006) where, polyamines prevent chlorophyll degradation in leaf discs of several higher plants. Other researchers added that, polyamines could prevent chlorophyll loss by stabilizing thylakoid membrane structure of oat leaf (Besford *et al.,* 1993), or by redistributing pigment protein complexes (Iordanov and Goltsev, 1990). The studies of Kao (1994) using 5 mM D-arginine and Chattopadhayay *et al.,* (2002) using putrescine, spermidine and spermine on maize and rice respectively demonstrated the retardation of leaf senescence via the retardation of chlorophyll loss.

The effect of polyamines on carbohydrate contents, Santa Cruze *et al.* (1998) observed that, application of putrescine was paralled to accumulation of sugars in leaves of tomato. However, Das *et al.* (2002) concluded that, foliar spray of spermidine (1mM) reduced the sugar contents of mulberry plants.

Polyamines are able to bind several negatively charged molecules, such as DNA (Pohjanpelto and Holtta, 1996), post transcriptional modifications (Mehta *et al.,* 1994) and conformational transition of DNA (Basu *et al.,* 1990). The increase in the levels of RNA and DNA by application of polyamines was reported by Chattopadhayay *et al.* (2002). Also, Tood and Gifford (2003) on pine found that, application of exogenous arginine to seedling caused an increase in total mRNA accumulation.

The increase in proline contents in plants treated with polyamines was recorded by (Nassar, 1997). El-Bassiouny (2004) observed an increase in proline and total soluble protein contents of *Pisum sativum* plants treated with putrescine. The same author also stated that, amino acids and polyamines are related in their metabolic pathways and affected by alteration in enzymatic levels. On the other hand, Mansour *et al.* (2002) and Das *et al.* (2002) observed that, polyamine treatments increased protein content but reduced proline accumulation of wheat and mulberry plants respectively. Vervaeke *et al.* (2005) stated that, the involvement of arginine was probably related to protein synthesis in *Aechmea fasciata* plant.

Chang and Kang (1999) working on nuclear protein of R*anunculus* petioles showed that, polyamine stimulated the phosphorylation of 17.5, 26, 30 and 35 KDa proteins. Spermine exhibited the most pronounced effect of protein phosphorylation among the polyamines tested for 17.5 and 35 KDa proteins. Moreover, putrescine treatment induced new proteins at M wts (157, 111, 55, 41 and 29 KDa) as compared with the control in pea shoot (El-Bassiouny, 2004).

Krishnamurthy (1991) reported that, foliar application of putrescine enhanced the uptake of K, Ca, Mg and P in rice plants. Moreover, Aziz *et al.* (1999) discussed the relation between polyamine contents and ions (K^+ and Ca^{+2}) in tomato leaf discs. They showed that, as Ca^{+2} ions increased the endogenous polyamines increased. Moreover, Mansour *et al.* (2002) showed that, polyamine application increased Ca and K contents in shoot and root of wheat plant.

The aim of the present work: is to study the effect of foliar application of arginine (as the precursor of polyamines) or putrescine at different spraying time on growth and yield components. The changes in the endogenous phytohormones including; auxins, gibberellins, cytokinins and growth inhibitors (ABA) in shoots of wheat plants were also investigated. The photosynthetic pigments of leaves, the carbohydrates contents, nucleic acid levels, nitrogenous components, protein electrophoratic pattern and element composition of wheat shoots were also studied.

MATERIALS AND METHODS

The experimental plant used in this investigation was wheat (*Triticum aestivum* var. Giza 168). Pure strain of grains obtained from Egyptian Ministry of Agriculture.

The chemicals used in the present work were (i) arginine (one of the essential amino acids), (ii) the diamine; putrescine (member of polyamine group). They were supplied from SIGMA – ALDRICH.

Two pot experiments were carried out in the screen of National Research Centre, Dokki, Giza, Egypt during two successive growth seasons (15 / 11 / 2003 and 2004) This experiment was carried out to study the effect of different concentrations of arginine or putrescine (0.0, 0.60, 1.25, 2.50 and 5.00 mM) as well as different dates of spraying, 30 days after sowing (vegetative), 60 days after sowing (just before emergence of spikes) and 90 days after sowing (during grain filling) on growth, biochemical changes and yield of wheat plants.

A homogenous lots of wheat grains *Triticum aestivum* var. Giza 168 were sown in pots (50 cm in diameter) containing equal amounts of clay soil. Fertilization was done with the recommended dose i.e. (5 g phosphorous / pot as triple phosphate, 6 g nitrogen / pot as urea and 5 g potassium / pot as potassium sulphate) during preparation of pots and after sowing. Watering was carried out according to the usual practice. After 15 days from sowing thinning was carried out, so as five uniform seedlings were left in each pot. Three plants from each pot were used through out the experimental period for biochemical analysis and the remaining two seedlings were left to grow for studying the effect of different treatments on the yield.

The pots were divided into three sets each composed of 90 pots. The pots of the first set were divided into 9 groups, each group composed of 10 pots. The plants of the 9 groups were sprayed with H_2O (as control), 0.60, 1.25, 2.50 and 5 mM arginine and 0.60, 1.25, 2.50 and 5.00 mM putrescine, respectively. These treatments were carried out twice at 30 and 37 days after sowing. In the meantime, the plants of the second set were also divided as the same manner of the first set and sprayed with the same concentrations twice at 60 and 67 days after sowing. The third set was divided into 9 groups as mentioned above and sprayed twice at 90 and 97 days after sowing. Samples of the first and second set were harvested at 75 days from sowing. While, the third set was left to yield.

The endogenous growth regulators, indole acetic acid (IAA), gibberellins (GA_s), abscisic acid (ABA), cytokinins as zeatin, zeatin riboside (ZR) and zeatin glucoside (ZG) were estimated in the fresh shoots. Photosynthetic pigments, nucleic acids and protein bands were also quantified in the fresh leaves. Carbohydrates, nitrogen fractions and mineral ion contents (potassium, calcium, magnesium and phosphorous) were determined in oven dried plant material.

Yield components of the 3 sets were recorded after 135 days from sowing. In addition, carbohydrate contents and nitrogen constituents were analyzed in the harvested grains.

Extraction, Separation and Determination of Phytohormones**:** The method of hormone extraction was essentially similar to that adopted by Wasfy and Orrin, (1975) and the Methylation process was carried out according to the method described by Vogel (1975). Identification and determination of auxins, gibberellins, and abscisic acid were carried out by Helwett Packered gas liquid chromatography (5890) with a flame ionization detector (Shindy and Smith, 1975). The gas liquid chromatographic conditions for isothermal work was as follows: The chromatograph was fitted and equipped with HP–130 mmX0.32 mm X 0.25 µm capillary column coated with methyl silicon. The column oven temperature was programmed at 10˚ C/min from 200˚ C (5 min) to 260˚C and kept finally to 10 min. Injector and detector temperatures were 260 and 300˚C, respectively. Gases flow rates were 30, 30, 300 cm/sec for N_2, H_2 and air, respectively and flow rate inside column was adjusted at 2 ml / min. Standards of IAA, GA_3 and ABA were used. Cytokinin fractions (zeatin, zeatin riboside and zeatin glucoside) were detected by HPLC isocratic UV analyzer ODS Hyparsil C18 column, 20 min gradient from 0.1N acetic acid. pH 2.8 to 0.1 N acetic acid in 95% aqueous ethanol, pH 4. The flow rate: 1ml/min, detection: UV 254 nm, standards of zeatin, zeatin riboside and zeatin glucoside were used (Muller and Hilgenberg, 1986).

The Photosynthetic Pigments (chlorophyll a, chlorophyll b and carotenoids) were determined spectrophotometrically by the method recommended by Metzner *et al.* (1965). Total soluble sugars and sucrose were determined by using the modified procedures of Yemm and Willis, (1954) and Handel (1968) respectively. Total carbohydrate content was determined colourimetrically according to Dubois *et al.* (1956) using Spekol Spectrocolourimeter VEB Carl Zeiss. The method used for the extraction of total RNA and DNA is that of Schmidt and Thannhauser, (1945) with some modifications as described by Morse and Carter, (1949).Ribonucleic acid (RNA) was estimated colourimetrically by the orcinol reaction as described by Dische, (1953). Deoxyribonucleic acid (DNA) was estimated by diphenylamine (DPA) colour reaction described by Burton, (1956). The nucleic acids were determination by Spekol Spectrocolourimeter VEB Carl Zeiss Free amino acids can be determined directly by the method of (Muting & Kaiser, 1963). Free proline was determined according to Bates *et al.* (1973).by Spekol Spectrocolourimeter VEB Carl Zeiss. The total soluble-N and total nitrogen were determined by the conventional micro-kjeldahl method (Pirie, 1955). Potassium,

calcium, magnesium and phosphorus were determined according to the method of Chapman and Pratt (1978). Determination of protein banding pattern: Protein extraction was done according to Reuveni *et al.* (1992) with some modifications. Electrophoretic protein profile of wheat shoots were analyzed according to sodium dodocylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) technique (Sheri, *et al.,* 2000). Polypeptide maps, molecular protein markers, percentage of band intensity, molecular weight and mobility rate of each polypeptide were related to standard markers using gel protein analyzer version 3 (MEDIA CYBERNE TICE, USA).

Statistical Analysis:

The results were statistically analyzed using MSTAT- C software. The mean comparisons among treatments were determined by Duncan's multiple range test at 5 % level of probability (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Growth Responses:

The foliar application of various concentrations (0.6, 1.25, 2.5 and 5 mM) of either arginine or putrescine decreased significantly shoot length of wheat plants sprayed at different stages of growth (vegetative, bolting and during grain filling) as compared with that of the control (Table 1). Generally, the magnitude of reduction increased by increasing the concentrations of both substances. On the other hand, the previous mentioned treatments induced significant increases in the number of tillers per plant, the number and area of wheat leaves per plant, the fresh and dry weight of shoots and roots of treated wheat plant compared to the control plants (Table 1). The results also show that spraying wheat plants at 30 DAS was most effective as compared with the same treatments applied at 60 DAS.Theses results were in harmony with those reported by Smith (1985) who showed that, polyamine concentrations were associated with short internodes through decreasing the internodes expansion. Also, he suggested that, PAs might be important in inducing cell division but not in inducing cell elongation. Vervaeke *et al.* (2005) showed that, higher concentrations of Put (2 and 5 mM) resulted in shorter pollen tubes of *Aechmea fasciata*.

Interestingly, the reduction in shoot length concomitantly with an increase in the circumference of stems parallel to the increase in arginine or putrescine concentrations could be achieved in response to the newly synthesized cytokinins and / or might be due to the more potent ABA. These results are in agreement with those obtained by Iqbal & Ashraf (2005) on wheat. The marked increase in the number of tillers per plant, the number and area of wheat leaves of treated wheat concurrently with the elevation of endogenous phytohormones in particular cytokinins in response to arginine or putrescine application (Table 3) lend a strong support to the results of El–Bassiouny (2004) and (Tang *et al.,* 2004) who indicated that application of Put increased significantly the branches number and endogenous cytokines of pea plant. So, the role of PAs in increasing tillers number may be via cytokinins and / or gibberellins which stimulate the mobilization of nutrient towards the buds through increasing cell divisions and / or increasing the differentiation of the vascular connection between the axillary buds and the main stem.

The increase in the fresh and dry weight of treated wheat plant is a reflection to the increase in growth rate (He–Lixiong *et al.*, 2002) cell division and / or cell enlargement and differentiation (Davies, 1995). In support of theses results Paschalidis & Roubelakis–Angelakis (2005) reported that, polyamines, their precursors (arginine) and their biosynthetic enzymes correlated with cell division, expansion, differentiation and development in tobacco plant. Couee *et al.* (2004) indicated that, the stimulation of polyamines to root growth and development may be related to the high flexibility of polyamine metabolism and the metabolic link between polyamine and ethylene synthesis which strongly suggest that, polyamines may play a role in environmentally induced plasticity of root development.

Yield Components:

The application of various concentrations of arginine or Put in the present work induced significant increases in the yield components of wheat plants represented by spikes number and weight per plant; grains number and weight per plant, 1000 – grain weight, biological yield per plant, harvest index and crop index (Tables 2). The magnitude of increase was much more pronounced; in most cases; by applying 2.5 mM of either arginine or Put. The results also showed that, exogenous application of both substances at 30 DAS was the most effective in increasing yield of wheat plant as compared with plant sprayed at 60 or 90 DAS.

The positive increases in the yield and its components in response to arginine or Put are in agreement with those obtained by Iqbal & Ashraf (2005) who applied Put on wheat plants. These increments in the yield

component due to arginine or Put treatments may be attributed to the increase in growth rate (Table 1). In this respect, Davies (1995), reported that polyamines play a critical role in different biological processes, including cell division, growth, somatic embryogenesis, floral initiation, development of flowers and fruits.

It is worthy to mention that, there is a close relationship between the effect of PAs and the stimulated growth, endogenous phytohormones, the photosynthetic output (soluble sugars, polysaccharides & total carbohydrates) and the nitrogen constituents (Tables 1, 3, 4). These results might increase the efficiency of solar energy conversion which maximized the growth ability of wheat plant and consequently increased its productivity and yield components. These results were strongly supported by those of Das *et al.* (2002) who found that, foliar spray of mulberry plants with PA increased the growth parameters, chlorophyll contents, protein contents as well as leaf yield as compared with control plant. The increases in the endogenous phytohormones (in particular cytokinins) in the present work increased the yield components through breaking the apical dominance of wheat plants leading to the increase in flowering tillers and consequently the number of spikes and their weight and/or through increasing the assimilates and their translocations from leaves to spikes as the spike weight increased. Gupta *et al.* (2003) illustrated that, Put increased grain yield, biological yield and seed weight index of wheat. They attributed these increments to the increases in chlorophyll content and transpiration rate.

Chemical Analysis of Yielded Grains:

The different treatments of arginine or putrescine effectively increased the total carbohydrate and protein percentage of yielded wheat grains over those of the untreated plant (Table 2). The magnitude of such response was much more pronounced by applying 2.5 mM of both arginine and Put Also, application of both substances at 30 DAS increased significantly the percentage of total carbohydrates and protein over those sprayed at 60 or 90 DAS (Table 2). These results may be attributed to the role of PAs in increasing the photoassimilates and subsequently the growth rate of wheat plants. These results agreed with those obtained by El – Bassiouny & Bekheta (2001) who showed that, external supply of Put increased the carbohydrate contents of wheat grains. Also El–Bassiouny (2004) demonstrated that, putrescine treatments significantly increased the protein percentage of the yielded pea seeds while carbohydrate percentage was not affected as compared with control. She attributed this increase to the translocation of amino acids from shoots to seeds and the increase in protein synthesis in pea shoot.

Endogenous Phytohormones:

It has been found in the present work that, the different treatments of arginine or putrescine effectively increased quantitatively the endogenous phytohormones such as IAA, GA_3 and cytokinins of the sprayed wheat plants at 30 or 60 days over those of the untreated plants (Tables 3). The most pronounced effect was observed in response to 2.5 mM of either arginine or putrescine. The results also show that, spraying wheat shoots at 30DAS induced higher significant increases in IAA, GA_3 and cytokinins over those plants treated with the same concentrations sprayed at 60DAS. In this respect, El–Bassiouny (2004) stated that, PAs increased the endogenous IAA, GA_3 and cytokinins content of pea plant.

The increases in IAA, GA_3 and cytokinins contents in shoot tissues treated with arginine or putrescine paralled with the increase in growth rate (Table 1) could be attributed to the stimulation in cell division and / or cell enlargement. Nassar (1997) recorded similar results in pea plants. In addition, putrescine treatments increased the content of cytokinins which in turn increased growth (number of branches and leaves) and endogenous IAA and GA of pea plant (El–Bassiouny, 2004).

The increases in the endogenous growth promoting substances in response to arginine or Put treatments might be attributed to their effect on increasing the biosynthesis of the endogenous growth promoters and / or decreasing their inactivation. The increase in the different growth promoters could be occurred through retarding the biosynthesis of hormone degradative enzymes and / or repressing their activities or through preventing the transformation of these active substances into inactive forms. These results agreed, in part, with those obtained by Nag *et al.* (2001) and Bekheta & El– Bassiouny (2005) who demonstrated that, Put increased the auxin content in treated plants due to retarding the destruction of endogenous auxins through decreasing the activity of IAA–oxidase of mung bean and wheat plants, respectively. In addition, Bais & Ravishankar (2003) showed that, Put treatment increased endogenous IAA of *Cichorium intybus* plants. PAs could induce their effect via affecting auxin availability through formation of conjugates with phenolics (Galston and Kaur – Sawhney, 1988). The outputs from the IAA pool via IAA oxidation include the regulation by phenols. Briefly, monophenols stimulate IAA–oxidation while p–diphenol, o–diphenol, coumarins and polyphenols are inhibitory.

The effect of Polyamines on increasing the endogenous cytokinins, Uperti & Murti (1999) and Bekheta & El–Bassiouny (2005) reported that, putrescine enhanced the cytokinins contents of pea and wheat plants, respectively.

The results of the present investigation showed that, arginine or putrescine treatment markedly decreased quantitatively ABA content of treated wheat plants. The most pronounced effect was observed by applying 2.5 mM arginine or putrescine. The reduction in ABA could be attributed to the antagonistic effect of PAs to ABA synthesis and / or action. This conclusion was reported by Smith (1985) who mentioned that, the application of PAs stimulate the a– amylase synthesis in aleurone layer which inhibited by ABA. This means that, PAs acting antagonistically to ABA action. Also, Nassar (1997) found that, endogenous ABA responses to the different arginine or Put treatments in either control or treated plants throughout the duration of the experiment were generally the reverse of those obtained with GA_3. However this result is logic on the bases of the participation of mevalonate as a common precursor in both gibberellin and ABA biosynthetic pathways (Walton, 1988). Recently, Iqbal *et al.* (2006) found that polyamines reduced the level of ABA and increased IAA content in wheat plant.

Photosynthetic Pigments:

The application of either arginine or Put increased the chlorophyll (a and b), carotenoids and consequently the total pigment contents significantly in the leaves of wheat plants as compared with the control (Table 3). The most effective concentration in this concern was 2.5 mM of either arginine or putrescine. Similar promoting effects of PAs on photosynthetic pigments had been observed by Das *et al.* (2002), Nassar *et al.* (2003) on mulberry and bean plants, respectively. A possible explanation for the promoting effect of arginine and putrescine on photosynthetic pigment of wheat plant in the present work is that PAs might retard the chlorophylls destruction and / or increase their biosynthesis or stabilize the thylakoid membrane. The role of polyamines in chlorophyll synthesis is supported by Askar–Treptow (1986) who suggested that; diamine aminotransferase specially transfers the amino group of Put to a - oxoglutaric acid which is the precursor of chlorophyll. Moreover, putrescine has several physiological attributes in increasing the chlorophyll contents of wheat plants where it was accompanied by an increase in endogenous cytokinins (Table 3). Cytokinins were found to stimulate chlorophyll biosynthesis and chloroplast differentiation in wheat plant (Xie *et al.*, 2004). Retention of photosynthetic pigments by PAs via interaction with thylakoid membrane was also stated by Gonzalez *et al.,* (1997). They also, demonstrated that, PAs may retard senescence via altering the stability and permeability of such membranes and protecting membranes and prevent chloroplast from senescing and therefore retarding chlorophyll loss. Moreover, Chattopadhayay *et al.* (2002) added that, PAs prevented chlorophyll loss, inhibition of photochemical reactions of photosynthesis and down – regulation of chloroplast – encoded genes in rice. Recently, HuiGuo *et al.* (2006) found that exogenous application of polyamines protects PSII against water stress at both transcriptional and translational levels and allow PSII to retain a higher activity level during stress, in wheat seedlings resulting in the increase in chlorophyll contents. In this connection, Kao (1994) found that, 5 mM D–arginine retarded senescence of maize.

The increases in chlorophyll contents as a result of arginine and Put treatments concomitantly with increasing in Mg levels (Table 4) 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 (Krishnamurthy, 1991 on rice).

The results of the present work also indicated a substantial increase of carotenoid levels in the arginine or putrescine treated plants which would be a further support to explain their higher content of chlorophylls. The protective role of carotenoids, as a photodynamic effect, is of a vital importance; it may be the primary function of the pigments (Krinsky, 1978). In addition, carotenoids act as photoreceptor pigment (Lawlor, 1989). It has been found in the present work that there is a positive correlation between the increase in growth rate, assimilating area and photosynthetic pigment contents in response to arginine or putrescine treatments in wheat plants. The magnitude of such response was much more pronounced by applying 2.5 mM of arginine or putrescine. The same observations were confirmed by He–Lixiong *et al.* (2002) and Nassar *et al.* (2003) using arginine or Put on cucumber and bean plants, respectively.

Carbohydrate Contents:

The present investigation showed that, arginine or Put treatments induced significant increases in sucrose, total soluble sugar, polysaccharide and total carbohydrate contents of treated wheat plants over those of the untreated plants (Table 3). These increments were closely correlated to the stimulation of leaves area chlorophyll biosynthesis, Mg contents and accumulation of dry matter in wheat shoots (Table1, 3 & 4). In this

connection, Rugini *et al.* (1993) recorded that, Put treatments at shoot proliferation phase induced a fully expanded leaves of both pear and *Pyrus calleryana*. Hopkins and Huner (2004) stated that, Mg is an activator for ribulosebisphosphate carboxylase (Ribusco), the critical enzyme in carbon dioxide fixation. These results are in agreement with those obtained by El– Bassiouny & Bekheta (2001) who stated that, exogenous application of Put induced significant increases in chlorophyll, Mg contents and total soluble sugar. Also, Liu *et al.* (2002) added that, application of PAs increased chlorophyll and soluble sugar contents of *Brassica campestris*. Sood and Nagar (2005) concluded that, the retarding effect of polyamines on leaf senescence of rose species was reflected in increasing the reserve metabolites as chlorophyll, protein and starch.

Nucleic Acid Contents:

DNA and RNA contents of wheat shoots (Table 4) were increased in response to arginine or putrescine treatments over those of the untreated plants. The most pronounced effect was observed in the plants exposed to 2.5 mM of both arginine and Put. Similar promoting effects of PAs were observed by other investigators who suggested that, DNA, RNA and protein contents were significantly higher in polyamines treated plants (Sood & Nagar, 2003; Couee *et al.,* 2004 and Bekheta & El– Bassiouny, 2005). Also, Tood & Gifford (2003) on pine found that, application of exogenous arginine (precursor of Put) to seedling caused an increase in total mRNA accumulation.

The increase in DNA and RNA in wheat plants in response to arginine or putrescine treatments could be attributed to the enhancement in their biosynthesis and / or inhibition in their degradation. The inhibition in DNA and RNA degradation might be ascribed to the role of polyamines in binding with DNA molecule or binding with RNase enzyme and hence preventing the hydrolysis of DNA or RNA. In support of these views, several investigators reported inhibition of RNAase enzyme activity in rice (Chattopadhayay *et al.,* 2002). However, Mehta *et al.*(1994) and Pohjanpelto and Holtta(1996) reported that, polyamines bind to nucleic acids and play an essential role in transcription, translation and protein synthesis. Moreover, PAs perform many physiological effects via the activation of the nucleic acids synthesis or by binding to negative charges of phospholipids and DNA, thereby stabilizing the function of the nucleus consequently promoting growth, cell division, DNA replication and cell differentiation (Tassoni *et al.,* 1996). In this respect, Papadakis and Roubelakis–Angelakis (2005) suggested that, PAs treatment could affect gene expression. They added that, Put increments might inhibit DNA methylation and in turn permitting the expression of specific genes. In *Chlamydomonas reinhardtii* the increase in PAs level during the transition from the cell enlargement to cell division was observed preceding the increase in DNA polymerase activity (Theiss *et al.,* 2002). In this respect, Paschalidis & Roubelakis – Angelakis (2005) indicated that, PAs synthesis has an essential role in cell division of *tobacco* plants.

Nitrogenous Constituents:

It has been found in the present investigation that, the different treatments of arginine or putrescine increased amino– N, proline, total soluble–N, protein– N and total N contents of wheat plants over those of control plants (Table 4). The high levels of nitrogenous contents in the shoots of the differently treated wheat plants concurrently with the active growth rate (Table 1) and the high contents of endogenous phytohormones, total carbohydrates, DNA and RNA (Table 3&4) could be attributed to the role of PA on enhancing protein synthesis and / or decreasing its degradation. In this respect Das *et al.* (2003) recorded positive increments in protein contents due to PA applications. In addition, Sood and Naggar (2003) suggested that, PAs act as activators to RNA, protein synthesis, and / or inhibit certain proteolytic enzymes. Guergue *et al.* (1997) added that, pretreatment with different concentrations of DL–alpha–difluoromethyl arginine (DFMA) an inhibitor of arginine decarboxylase activity) decreased callus protein of maize. Moreover, Ohlund and Nasholm (2001) illustrated that, 100 % of arginine in pine seedlings was derived from the uptake of intact amino acids (especially arginine) through seedling roots and concluded that, arginine act as N sources for growth. Moreover, Chang *et al.* (2005) demonstrated that, arginine (rich intracellular peptide) is capable of efficiently delivering proteins into different plant tissues of both tomato and onion in a fully bioactive form. Also, Vervaeke *et al.* (2005) stated that, the involvement of arginine was probably related to protein synthesis in *Aechmea fasciata* plant.

Regarding the role of PAs on stimulation of proline contents in wheat plants, Smith & Wood (1992) reported that, the increases in proline contents in plants treated with PAs may be refer to the catabolic processes of PAs to pyrroline which can be reduced into proline. In this respect, Nassar (1997) and El–Bassiouny (2004) indicated that, application of Put induced significant increases in proline contents of pea plants over control. Moreover, Kesba (2005) found that, foliar application of L – arginine (the precursor of putrescine) mainly enhanced the levels of proline in grape roots.

Table 1: Effect of foliar treatment with arginine or putrescine at 30 or 60 days after sowing on growth parameter of wheat plants at 75 days after sowing.

Parameter	Time of foliar treatments (days after sowing)	Control (0.00)	Arginine (mM)				Putrescine (mM)			
			0.60	1.25	2.50	5.00	0.60	1.25	2.50	5.00
Shoot length (cm)	30	77.30^{a}	61.75^{h}	55.35^{k}	52.75^{m}	47.80^{n}	68.70^{d}	63.42^{g}	56.65^{j}	42.50^{o}
	60		70.60^{c}	66.00^{f}	60.30^{i}	54.60^{l}	76.13^{b}	70.80^{c}	67.20^{e}	63.20^{g}
Number of tillers/plant	30	4.2^{d}	7.0^{b}	6.0^{c}	5.3c^{d}	4.9^{cd}	4.7^{d}	5.6^{c}	7.8^{a}	7.0^{b}
	60		5.2^{cd}	4.9^{cd}	4.6^{d}	4.4^{d}	4.4^{d}	4.8^{d}	4.9^{cd}	5.0^{cd}
Number of leaves/plant	30	20.5^{h}	35.0^{a}	30.2^{c}	26.5^{de}	24.5^{fg}	21.3^{h}	25.8^{ef}	32.9^{b}	30.8^{c}
	60		32.6^{b}	26.3^{de}	24.0^{g}	21.5^{h}	20.9^{h}	23.5^{g}	25.4^{ef}	27.5^{d}
Leaves area/ Plant(cm^{2})	30	155.50^{i}	282.5^{a}	247.6^{cd}	206.0^{ef}	196.3^{g}	170.3^{h}	204.3^{ef}	283.3^{a}	267.8^{b}
	60		260.8^{bc}	218.5^{e}	193.8^{fg}	173.0^{h}	159.8^{i}	189.5^{g}	238.5^{d}	214.3^{e}
Circumferance of main stem (cm)	30	1.15^{f}	1.65^{c}	1.80^{b}	2.02^{a}	1.83^{b}	1.42^{d}	1.55^{cd}	1.65^{c}	1.47^{d}
	60		1.45^{d}	1.52^{d}	1.55^{cd}	1.48^{d}	1.19^{ef}	1.40^{d}	1.48^{d}	1.28^{e}
Fresh weight of Shoot/plant(g)	30	6.82^{m}	15.46^{a}	14.94^{b}	12.47^{e}	11.10^{h}	10.18^{j}	13.37^{d}	15.14^{b}	15.05^{b}
	60		14.16^{c}	13.55^{d}	11.61^{g}	10.16^{j}	8.271	9.50^{k}	10.87^{i}	11.90^{f}
Dry weight of Shoot/plant(g)	30	1.68^{q}	3.49^{a}	2.94^{f}	2.62^{i}	2.23^{m}	2.09^{o}	3.00^{e}	3.28^{c}	3.31^{b}
	60		3.28^{d}	2.85^{g}	2.48^{k}	2.19^{n}	1.91^{p}	2.312^{l}	2.59^{j}	2.84$_{h}$
Fresh weight of root/plant(g)	30	0.417^{o}	0.514^{j}	0.777^{e}	0.842^{c}	0.926^{a}	0.564^{i}	0.596^{h}	0.753^{f}	0.793^{b}
	60		0.448^{m}	0.686^{g}	0.794^{d}	0.868^{b}	0.403^{p}	0.436^{n}	0.488^{l}	0.494^{k}
Dry weight of root/plant(g)	30	0.099^{q}	0.370^{h}	0.423^{e}	0.473^{c}	0.513^{a}	0.244^{n}	0.288^{l}	0.325^{i}	0.387^{g}
	60		0.264^{m}	0.394^{f}	0.441^{d}	0.492^{b}	0.216^{p}	0.237^{o}	0.294^{k}	0.309^{g}

Table 2: Effect of foliar treatment with arginine or putrescine at 30, 60 or 90 days after sowing on yield components of wheat plants.

Parameter	Time of foliar treatments (days after sowing)	Control (0.00)	Arginine (mM)				Putrescine (mM)			
			0.60	1.25	2.50	5.00	0.60	1.25	2.50	5.00
Plant height (cm)	30	88.7^{a}	72.8^{ghi}	67.25^{jkl}	66.6^{jkl}	61.8^{m}	79.6^{c-f}	77.3^{efg}	72.1^{hi}	64.4^{klm}
	60		75.0^{fgh}	69.8^{ij}	68.9^{ijk}	64.3^{lm}	82.0^{b-e}	80.6^{b-e}	79.8^{cde}	75.1^{fgh}
	90		82.5^{bcd}	82.1^{b-e}	79.7^{cde}	77.8^{def}	85.2^{ab}	83.6^{bc}	28.0^{b-e}	81.2^{b-e}
Tillers number /plant	30	3.5^{j}	6.0^{abc}	5.7^{a-e}	4.7^{e-i}	4.2^{hij}	5.1^{c-h}	6.0^{abc}	6.3^{ab}	6.5^{a}
	60		5.1^{c-h}	5.5^{a-f}	4.5^{f-j}	4.0^{ij}	4.8^{d-i}	5.3^{b-g}	5.8^{a-d}	6.0^{abc}
	90		4.2^{hij}	4.4^{g-j}	4.5^{f-j}	4.0^{ij}	4.5^{f-j}	4.8^{d-i}	5.0^{c-i}	5.2^{c-h}
Spikes number plant	30	3.0^{i}	5.3^{ab}	4.8^{b-e}	4.5^{d-g}	4.0^{gh}	4.7^{cde}	5.1^{abc}	5.5^{a}	5.0^{a-d}
	60		4.8^{bcd}	4.5^{d-g}	4.2^{efg}	4.0^{gh}	4.5^{d-g}	4.8^{bcd}	5.0^{a-d}	4.6^{c-f}
	90		4.0^{gh}	4.2^{efg}	4.0^{gh}	3.6^{h}	4.1^{fgh}	4.5^{d-g}	4.8^{bcd}	4.0^{gh}
Grain number/plant(g)	30	118.9^{k}	219.9^{b}	231.5^{b}	268.8^{a}	220.4^{b}	179.8^{def}	200.2^{c}	220.9^{b}	188.2^{cde}
	60		153.9^{hi}	187.6^{c-f}	218.8^{b}	161.4^{ghi}	143.9^{ij}	169.5^{fgh}	196.7^{cd}	154.7^{hi}
	90		132.0^{jk}	148.7^{ij}	174.6^{efg}	155.8^{hi}	123.1^{k}	149.4^{ij}	160.2^{ghi}	120.4^{k}
1000 - grains weight(g)	30	37.48^{l}	40.47^{g-j}	41.30^{def}	41.82^{bcd}	39.99^{ij}	40.80^{fgh}	42.26^{ab}	42.63^{a}	41.48^{cde}
	60		40.24^{hij}	40.62^{ghi}	41.13^{efg}	39.22^{k}	40.53^{g-j}	41.55^{cde}	42.16^{ab}	41.28^{def}
	90		39.88^{j}	40.77^{fgh}	41.33^{def}	39.09^{k}	40.20^{hij}	42.05^{abc}	42.27^{ab}	40.72^{fgh}
Straw yield/plant(g)	30	7.95^{ghi}	11.80^{a}	11.31^{ab}	10.94^{b}	11.90^{a}	8.33^{e-h}	8.05^{gh}	7.72^{hi}	7.68^{hi}
	60		9.26^{c}	9.04^{cde}	8.88^{c-f}	9.09^{cd}	8.61^{c-g}	8.89^{c-f}	8.67^{c-g}	8.97^{c-f}
	90		8.68^{c-g}	8.79^{c-f}	8.39^{d-h}	7.98^{ghi}	8.82^{c-f}	8.27^{fgh}	6.85^{i}	7.28^{ij}
Harvest index (%)	30	36.90^{l}	37.70^{k}	40.91^{fg}	45.00^{d}	40.35^{gh}	41.46^{f}	46.01^{c}	49.77^{a}	47.29^{b}
	60		34.88^{o}	40.37^{gh}	44.95^{d}	43.15^{e}	35.21^{no}	39.44^{i}	43.22^{e}	36.61^{l}
	90		35.80^{mn}	36.35^{lm}	41.53^{f}	38.52^{j}	35.76^{mn}	38.65^{j}	44.49^{d}	40.03^{hi}
Crop index (%)	30	58.49^{n}	60.51^{m}	69.23^{h}	81.81^{d}	67.65^{i}	70.83^{g}	85.22^{c}	99.09^{a}	89.71^{b}
	60		53.56^{r}	67.70^{i}	81.64^{d}	75.91^{f}	54.36^{q}	65.13^{k}	76.12^{f}	57.75^{no}
	90		55.76^{p}	57.11^{o}	71.04^{g}	62.66^{l}	55.67^{p}	63.00^{l}	80.15^{e}	66.76^{j}
Carbohydrate (%)	30	48.21^{gh}	50.97^{c-f}	52.55^{bc}	55.36^{a}	51.37^{cde}	49.33^{e-h}	51.84^{cd}	54.14^{ab}	49.95^{d-h}
	60		48.21^{gh}	49.00^{fgh}	51.17^{c-f}	48.65^{gh}	48.50^{gh}	48.71^{gh}	49.54^{e-h}	48.48^{gh}
	90		49.80^{d-h}	50.30^{d-g}	52.90^{bc}	49.70^{d-h}	48.03^{h}	51.40^{cde}	53.04^{bc}	49.13^{fgh}
Protein (%)	30	14.62^{m}	15.31^{g-k}	16.16^{bc}	16.63^{a}	15.78^{cf}	15.38^{f-g}	15.91^{b-e}	16.25^{ab}	15.19^{h-l}
	60		14.84^{lm}	15.00^{j-m}	16.00^{bcd}	14.63^{m}	14.66^{m}	14.88^{klm}	15.59^{d-h}	14.73^{lm}
	90		15.06^{i-m}	15.50^{e-i}	15.88^{b-e}	15.68^{d-g}	15.00^{j-m}	15.38^{f-j}	16.03^{bcd}	15.00^{j-m}

Mineral Composition:

The present investigation showed that, arginine or Put treatments induced significant increases in K, Ca, Mg and P contents in shoots of wheat plants compared to those of the untreated control plants (Table 4). In this respect, Grego *et al.* (1992*)* found that, PA treatments induced increases in K transport to the upper part of plant and enhanced translocation into growing tissues in maize seedlings. Suleiman *et al.* (2002) added that, PAs regulate the Ca^{2+} nutritional status of plant which important in maintaining selectivity and in turns the integrity of cellular membranes. Mansour *et al.,* (2002) and Iqbal & Ashraf (2005) reported that, priming wheat with PA (Put) enhanced the accumulation of K^{+} and Ca^{2+} in plant.

Table 3: Effect of foliar treatment with arginine or putrescine at 30 or 60 days after sowing on phytohormone, photosynthetic pigments and carbohydrate contents of wheat plants at 75 days after sowing.

Parameter	Time of foliar treatments (days after sowing)	Control (0.00)	Arginine (mM)				Putrescine (mM)			
			0.60	1.25	2.50	5.00	0.60	1.25	2.50	5.00
Endogenous phytohormones (µ g/100g fresh weight)										
IAA	30	11.43^{p}	24.83^{l}	44.21^{e}	66.44^{a}	53.8^{c}	28.61^{j}	39.06^{h}	56.84^{b}	42.47^{f}
	60		19.75^{o}	31.18^{i}	49.39^{d}	40.08^{g}	19.98^{o}	23.18^{m}	25.19^{k}	22.82^{n}
ABA	30	15.33^{a}	9.51^{d}	6.31^{i}	4.08^{k}	7.01^{h}	8.87^{e}	3.67^{l}	1.70^{n}	2.70^{m}
	60		13.42^{b}	8.31^{f}	7.36^{g}	10.29^{c}	9.10^{e}	4.47^{j}	3.43^{l}	6.39^{i}
GA_3	30	46.12^{kl}	52.61^{e}	59.00^{c}	68.66^{a}	60.66^{b}	48.22^{j}	50.64^{g}	65.79^{d}	51.22^{f}
	60		48.61^{ij}	49.64^{h}	52.56^{e}	45.72^{l}	46.32^{k}	48.84^{i}	52.54^{e}	48.64^{ij}
Cytokinins	30	41.04^{n}	57.71^{l}	78.15^{h}	87.12^{e}	70.23^{j}	85.81^{f}	96.09^{c}	116.35^{a}	88.47^{d}
	60		53.46^{m}	62.30^{k}	69.00^{j}	53.17^{m}	74.11^{i}	81.20^{g}	97.84^{b}	82.11^{g}
Photosynthetic pigments (mg/100g dry weight)										
Chl a	30	1268^{j}	1571^{h}	1593^{g}	1612^{f}	1444^{k}	1478^{j}	1533^{i}	1687^{cd}	1544^{i}
	60		1639^{e}	1678^{d}	1762^{b}	1635^{e}	1694^{c}	1755^{b}	1786^{a}	1638^{e}
Chl b	30	537^{i}	541^{i}	587^{f}	614^{e}	546^{i}	584^{fg}	628^{d}	682^{b}	575^{gh}
	60		572^{h}	593^{f}	645^{c}	565^{h}	594^{f}	648^{c}	727^{a}	591^{f}
Carotenoids	30	327^{l}	435^{j}	453^{i}	482^{h}	416^{k}	423^{k}	488^{gh}	540^{cd}	502^{f}
	60		493^{fg}	539^{cd}	563^{b}	544^{c}	500^{f}	533^{de}	589^{a}	524^{e}
Carbohydrate contents (mg/100g dry weight)										
Soucrose	30	817^{m}	950^{l}	1011^{k}	1272^{h}	1315^{f}	1024^{k}	1205^{i}	1392^{c}	1281^{gh}
	60		1195^{i}	1294^{g}	1360^{d}	1410^{b}	1178^{j}	1371^{d}	1498^{a}	1335^{e}
Total soluble sugar	30	2728^{i}	2862^{h}	3065^{f}	3294^{c}	3000^{g}	2989^{g}	3170^{e}	3327^{c}	3000^{g}
	60		2964^{g}	3193^{de}	3495^{a}	3167^{e}	3069^{f}	3226^{d}	3421^{b}	3168^{e}
Polysaccharides	30	15601^{n}	16479^{m}	17818^{i}	21212^{d}	18538^{g}	16775^{l}	18179^{h}	21547^{c}	19292^{f}
	60		17421^{j}	18102^{i}	22713^{a}	18463^{g}	17196^{k}	19497^{f}	21812^{b}	20133^{e}
Totel Carbohydrate	30	18329^{q}	19341^{p}	20883^{l}	24506^{d}	21538^{i}	19764^{o}	21349^{j}	24874^{c}	22293^{h}
	60		20385^{m}	21301^{k}	26208^{a}	22630^{g}	20265^{n}	22722^{f}	25233^{b}	23301^{e}

Table 4: Effect of foliar treatment with arginine or putrescine at 30 or 60 days after sowing on nitrogenous constituents,nucleic acids and mineral contents of wheat plants at 75 days after sowing.

Parameter	Time of foliar treatments (days after sowing)	Control (0.00)	Arginine (mM)				Putrescine (mM)			
			0.60	1.25	2.50	5.00	0.60	1.25	2.50	5.00
Nitrogenous content (mg/100g dry weight)										
Amino-N	30	116.66^{n}	348.72^{b}	247.90^{e}	230.90^{g}	120.82^{n}	154.16^{m}	169.99^{k}	199.99^{i}	162.50^{l}
	60		366.65^{a}	314.57^{c}	270.82^{d}	172.9^{k}	186.65^{j}	207.07^{h}	234.57^{f}	196.24^{i}
Proline	30	31.90^{j}	40.67^{d}	43.59^{bc}	46.69^{a}	41.41^{cd}	35.00^{ghi}	43.93^{abc}	46.31^{ab}	38.69^{def}
	60		35.83^{fgh}	38.69^{def}	41.30^{cd}	37.38^{efg}	33.45^{hij}	35.47^{gh}	39.17^{de}	32.38^{ij}
Total soluble-N	30	650^{i}	750^{f}	900^{b}	1100^{a}	850^{c}	700^{g}	760^{e}	900^{b}	750^{f}
	60		750^{f}	900^{b}	1100^{a}	850^{c}	700^{g}	760^{e}	900^{b}	750^{f}
Protein-N	30	2617^{l}	3917^{b}	3567^{d}	3000^{h}	2750^{k}	4033^{a}	3640^{c}	3349^{f}	3250^{g}
	60		3433_{e}	3000^{h}	2833^{j}	2650^{l}	2920^{i}	2800^{jk}	2667^{l}	2450^{m}
Totel-N	30	3267^{m}	4667^{b}	4467^{c}	4000^{g}	3550^{jk}	4733^{a}	4400^{d}	4199^{e}	4000^{g}
	60		4133^{f}	3800^{h}	3733^{i}	3400^{j}	3600^{j}	3500^{k}	3367^{l}	3100^{n}
Nucleic acids (µg/ 100g fresh weight)										
DNA	30	8.4^{j}	14.1^{g}	21.2^{c}	26.8^{a}	18.0^{e}	11.7^{h}	18.2^{e}	18.8^{de}	16.4^{f}
	60		12.4^{h}	19.6^{d}	22.5^{b}	16.3^{f}	8.9_{ij}	9.5^{i}	16.9^{f}	14.9^{g}
RNA	30	463^{l}	510^{fg}	567^{c}	619^{a}	565^{c}	486^{ij}	534^{d}	589^{b}	484^{ij}
	60		490_{ij}	505_{gh}	529^{de}	480^{jk}	469_{kl}	495^{hi}	520^{ef}	471_{kl}
Mineral contents (mg/100g dry weight										
K	30	2371^{n}	2866^{e}	3028^{b}	3128^{a}	2952^{d}	2671^{h}	2961_{c}	3132^{a}	2680^{g}
	60		2401^{m}	2500^{j}	2872^{e}	2462^{k}	2450^{l}	2642^{i}	2820^{f}	2365^{n}
Ca	30	1131^{l}	1386^{j}	1460^{h}	1542^{d}	1433^{i}	1359^{k}	1473^{g}	1565^{c}	1488^{f}
	60		1433^{i}	1523^{e}	1629^{a}	1491^{f}	1485^{f}	1590^{b}	1632^{a}	1563^{c}
Mg	30	483^{n}	494^{m}	531^{j}	593^{e}	519^{k}	508^{l}	568^{g}	634^{c}	589^{ef}
	60		523^{k}	583^{f}	665^{b}	560^{h}	538^{i}	595^{e}	685^{a}	613^{d}
P	30	1412^{n}	1889^{g}	2095^{c}	2193^{b}	1917^{f}	1533^{m}	1768^{k}	1918^{f}	1704^{l}
	60		1988^{e}	2179^{b}	2365^{a}	2044^{d}	1811^{j}	1853^{h}	1988^{e}	1831^{i}

The increase in element contents concurrently with the active growth rates of wheat shoots, in the present work, led to the suggestion that, the exogenous application of PAs could indicate their involvements in the maintenance of these element levels to enhance the metabolic processes in which these nutrients are utilized. In this connection, Sharma *et al,.* (1997) and El–Bassiouny and Bekheta (2001) revealed that, foliar application of Put enhanced the uptake of K, Ca, Mg and P of rice, chick pea and wheat plants, and illustrated the role of Mg in some metabolic processes. Moreover, other investigators revealed that, the main role of PAs in plants, in long term, is to maintain cation / anion balance in plant tissue (Mansour & Al – Mutawa, 1999).

Protein Electrophoretic Pattern:

The electrophoretic pattern of proteins in wheat shoots in response to different treatments of arginine or putrescine showed a striking difference compared with the untreated plants. The treatments induced the disappearance of certain bands and the appearance of new ones as compared with that of the untreated plants (Table 5). In this respect, Mizrahi *et al.* (1989) concluded that, PAs are important cellular constituents to specific regulatory proteins. They provide a possible mechanism for the formation of polyamine – protein complexes. Kuznetsov and Shevyakova (1997) stated that, PAs could change the stability and substrate specificity of protein kinase / phosphatase systems to modify the properties of polypeptides and acting as substrates for phosphorylation and dephosphorylative enzymes and affect the stability of protein molecules in plants.

It is worthy to mention that, arginine or putrescine treatments induced the appearance of new proteins at M wts. 222.7, 214.6, 131.8, 93.1, 78.7, 50.7, 34.6 and 14.1 KDa respectively (Table 5). These results are in agreements with those obtained by Datta *et al.* (1987) who found that, PAs regulate protein phosphorylation of one or more polypeptides which have an apparent molecular mass of 47 KDa in isolated and intact pea nuclei. Also, Chang and Kang (1999) stated that, PAs stimulated the phosphorylation of 17, 26, 30 and 35 KDa of petioles of *Rannuculus* plant. EL–Bassiouny (2004) revealed that, the application of Put on pea plants induced the synthesis of new proteins at M wts. 157, 111, 55, 41 and 29 KDa. Moreover, Bekheta and El–Bassiouny (2005) indicated that, Put treatments induced the appearance of new set of protein in two wheat cultivars at molecular weights 309, 91, 70, 36, 21 and 17 Kda.

Table 5: Effect of foliar treatment of arginine or putrescine at 30 or 60 days after sowing on molecular weights and relative area (%) of each protein band.

Bands number	M wt.	Control	Sprayed at 30 days after sowing								Sprayed at 60 days after sowing							
			Arginine (mM)				Putrescine (mM)				Arginine (mM)				Putrescine (mM)			
			0.60	1.25	2.50	5.00	0.60	1.25	2.50	5.00	60	60	60	60	60	60	60	60
1	222.7	-------	6.72*	10.61*	15.25*	29.50*	15.84*	0.76*	4.59*	15.67*	-------	-------	-------	-------	-------	-------	-------	-------
2	214.6	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	14.33*	7.59*	10.62*	-------	9.60*	18.33*	11.92*
3	158.0	6.287	-------	-------	4.78	4.26	22.34	13.53	10.56	-------	14.19	-------	9.45	-------	6.25	-------	6.22	11.73
4	131.8	-------	23.95*	7.88*	-------	-------	-------	-------	-------	-------	1.96*	11.02*	12.69*	4.31*	-------	4.61*	-------	-------
5	105.0	3.147	-------	-------	-------	-------	-------	-------	-------	4.98	-------	11.19	-------	22.36	5.68	7.15	4.88	8.88
6	93.1	-------	9.59*	5.97*	5.46*	6.30*	11.45*	15.87*	14.38*	17.07*	-------	-------	-------	-------	-------	-------	-------	-------
7	88.5	2.068	-------	-------	-------	-------	-------	-------	-------	-------	16.55	9.83	9.06	26.86	8.50	4.67	5.79	18.33
8	78.7	-------	-------	-------	5.656*	8.029*	13.929*	7.813*	20.667*	23.34*	-------	-------	-------	-------	-------	-------	-------	-------
9	68.0	5.426	16.393	6.888	-------	-------	-------	-------	-------	-------	15.24	9.53	7.95	8.24	30.18	31.25	25.40	29.14
10	50.7	-------	18.32*	52.61*	17.50*	10.64*	16.06*	34.95*	4.16*	8.69*	-------	-------	-------	-------	-------	-------	-------	-------
11	38.2	58.731	5.67	3.95	25.02	18.14	8.90	5.78	12.90	6.29	22.20	18.03	16.05	8.46	26.13	26.20	22.89	15.28
12	34.6	-------	-------	-------	-------	-------	-------	-------	-------	-------	16.19*	12.202	10.72*	9.52*	21.77*	16.52*	16.49*	4.71*
13	30.5	15.789	-------	-------	-------	-------	-------	-------	-------	-------	8.64	9.89	9.02	5.50	-------	-------	-------	-------
14	22.6	2.552	10.97	6.71	9.67	5.98	-------	12.71	14.51	9.43	5.04	3.98	17.47	4.14	1.49	-------	-------	-------
15	14.1	-------	8.40*	5.39*	16.66*	17.16*	11.49*	8.58*	18.23*	14.53*	-------	-------	-------	-------	-------	-------	-------	-------
Bands number		7	8	8	8	8	7	8	8	8	8	9	9	9	7	7	7	7
numbers of new Bands		-------	5	5	5	5	5	5	5	5	2	3	3	3	1	3	2	2

Concerning arginine effect on the electrophoretic pattern of protein in wheat shoots, Borrell *et al.* (1996) detected a dramatic decrease in the levels of 24 KDa band and a progressive accumulation of polypeptide at 66 KDa by exogenous application of PAs, and cleared that, in oat arginine decarboxylase (ADC) is synthesized as a pre–protein at 66 KDa which is cleaved to produce a fragment of 42–KDa and 24–KDa polypeptides. Thus, the 24-KDa band detected in protein gel represents the processed from the ADC enzyme, while the 66 – KDa band may represent the precursor ADC protein (Borrell *et al.,* 1995).

Finally, we can concluded that, spraying plants at the vegetative stage (30 DAS) and with 2.5 mM of either arginine followed by putrescine could be considered the most suitable time and the most suitable concentration to expect promising improvements regarding the growth parameters, physiological characters of wheat plants and enhancement the yield quantity and quality.

Abbreviations:

Days after sowing, DAS; Putrescine, Put; Harvest index, HI; Crop index, CI; Indole acetic acid, IAA; Gibberellins, $GA_{3;}$ Abscisic acid, ABA.

REFERENCES

Askar, A. and H. Treptow, 1986. Biogene amine in lebensmittlen a Veleg Eugen ulmer, Stuttgart.

Aziz, A., J. Martin-Tanguy and F. Larher, 1999. Salt stress – induced proline accumulation and changes in tyramine and polyamine levels are limited to ionic osmotic adjustment in tomato leaf discs. Plant Sci., 145: 83-91.

Bais, H.P. and G.A. Ravishankar, 2003. Synergistic effect of auxins and polyamines in hairy roots of *Cichorium intybus* L. during growth, coumarin production and morphogenesis. Acta Physiol. Planta, 25(2): 193 – 208.

Basu, H.S., H.C.A. Schwietert, B.C. Feuerstein and L.J. Marton, 1990. Effect of variation in the structure of spermine on the association with DNA and the induction of DNA conformational changes. Biochem. J., 269: 329-334.

Bates, L.S., R.P. Waldren and I.D. Tear, 1973. Rapid determination of free proline for water – stress studies. Plant and Soil, 39: 205 – 207.

Besford, R.T., C.M. Richardson, J.L. Campos and A.F. Tiburcio, 1993. Effect of polyamines on stabilization of molecular complexes in thylakoid membranes of osmotically stress oat leaves. Planta, 189(62): 201-206.

Borrell, A., F.A. Culiaez-Macia, T. Altabella, R.T. Besford, D. Flores and A.F. Tiburcio, 1995. Arginine decarboxylase is localized in chloroplasts. Plant Physiol., 109: 771–776.

Borrell, A., R.T. Besford, T. Altabella, C. Masgrau and A.F. Tiburcio, 1996. Regulation of arginine decarboxylase by spermine in osmotically – stressed oat leaves. Physiol. Plant, 98: 105 – 110.

Burton, K., 1956. A study of the conditions and mechanism of the diphenylamine reaction of colorimetric estimation of deoxyribonucleic acid. Biochem. J., 62: 315.

Chang, M., J.C. Chou and H.J. Lee, 2005. Cellular internalization of fluorescent proteins via arginine – rich intracellular delivery peptide in plant cells. Plant and Cell Physiol., 46(3): 482 – 488.

Chang, S.C. and B.G. Kang, 1999. Effect of spermine and plant hormones on nuclear protein phosphorylation in *Ranunculus* petioles. J. Plant Physiol., 154: 463 - 470.

Chapman, H.O. and P.E. Pratt, 1978. Methods of Analysis for Soils, Plants and Water. Univ. of California Agric. Sci. Priced Publication, 4034. pp: 50.

Chattopadhayay, M.K., B.S. Tiwari, G. Chattopadhyay, Anindica D.N. MBose Sengupta, G. Bharati, G. Chattopadhyay, A. Bose and B. Gosh, 2002. Protective role of exogenous polyamines on salinity stressed rice (*Oryza sativa*) plants. Physiol. Plant, 116: 192–199.

Couee, I., I. Hummel, C. Sulmon, G. Gouesbet and A. El-Amrani, 2004. Involvement of polyamines in root development. Netherlands, 76(1): 1-10.

Das, C., T. Sengupta, S. Chattopadhyay, M. Setua, N.K. Das and B. Saratchandra, 2002. Involvement of kinetin and spermidine in controlling salinity stress in mulberry (*Morus alba* L. cv. S1). Acta Physiol. Planta, 24(1): 53 – 57.

Datta, N., M.B. Schell and S.J. Rouz, 1987. Spermine stimulation of a nuclear N II kinase from pea plummules and its role in the phosphorylation of nuclear polypeptide. Plant Physiol., 84: 1397 - 1401.

Davies, P.J., 1995. Plant Hormones: Physiology and Biochemistry and Biology P. 159Kluwer Academic Publishers, London.

Dische, E.L., 1953. J. Amer. Chem. Soc. 22:3014. In Physiological studies on the herbicide "Cotorane" S.S. Roushdy, 1983. M. Sc. Thesis, Ain Shams Univ. Cairo Egypt.

Dubois, M., K.A. Gilles, J.K. Hamilton and P.A. Robers, 1956. Colourimetric method for determination of sugars and related substances. Anal. Chem., 28: 350–356.

El-Bassiouny, H.M.S., 2004. Increasing thermotolerance of *Pisum sativum* L. plants through application of putrescine and stigmasterol. Egypt. J. Biotech., 18: 93-118.

El- Bassiouny, H.M.S. and M.A. Bekheta, 2001. Role of putrescine on growth, regulation of stomatal aperture, ionic contents and yield by two wheat cultivars under salinity stress. Egyptian J. Physiol. Sci., 2-3: 235-258.

El-Bassiouny, H.M.S. and M.A. Bekheta, 2005. Effect of salt stress on relative water content, lipid peroxidation, polyamines, amino acids and ethylene of two wheat cultivars. Int. J. Agric. Biol., 7(3): 363-368.

Glaston, A.W. and R. Kaur – Sawhney, 1988. Polyamines as endogenous growth regulators. In: Plant Hormones and their Role in Plant Growth and Development, pp: 280 – 295. P. J. Davies, ed. Kluwer Academic Pub. Dordrecht, the Netherlands.

Glaston, A.W. and R. Kaur - Sawhney, 1995. Polyamines as endogenous growth regulators- In Plant Hormones: Physiology, Biochemistry and Molecular Biology. 2nd Ed.(P. J. Davis, ed), pp: 158- 178. Kluwer Academic Publishers, Dordrecht ISBNO.

Gomez, K.A. and A.A. Gomez, 1984. Statistical procedures for agricultural research. New York: John Wiley and Sons Publication., pp: 460.

Gonzalez – Aguilar, G.A., L. Zacarias, M. Mulas and M.T. Lafuente, 1997. Temperature and duration of water dips influence chilling injury, decay and polyamine content in Fortune mandarins Postharv Biol. Technol., 12: 61 – 69.

Grego, S., F. De – Cesare and M. De – Agazio, 1992. Exogenous spermidine as a tool to investigate a possible role for internal K^+ ions in cutting injury. Plant Physiol. Biochem. Paris., 30:(5) 593 – 596.

Guergue, A., I. Claparols, M. Santos and J.M. Torne, 1997. Modulator effect of DL–α–difluromethylarginine treatments on differentiation processes of yong maize calluses. Plant Growth Regul., 21: 7 – 14.

Gupta, S., M.L. Sharma, N.K. Gupta and A. Kumar, 2003. Productivity enhancement by putrescine in wheat (*Triticum aestivum* L.). Physiol. Mol. Biol. Plants, 9:(2) 279 – 282.

Handel, E.V., 1968. Direct microdetermination of sucrose. Anal. Biochem., 22: 280.

He–Lixiong, K. Nada and S. Tachibana, 2002. Effects of spermidine pretreatment through the roots on growth and photosynthesis of chilled cucumber plants *Cucumber sativus* L. J. Japan. Soc. Hort. Sci., 71: 490 - 498.

Hopkins, W.G. and N.P.A. Hüner, 2004. Introduction to Plant Physiology. 3rd Edition. John Wiley and Sons, Inc.

HuiGuo, D., Y. Shu, L. WenJuan, X. DeHui, Q. DongHong, l. HouGuo and HongHui, 2006. Effects of exogenous spermidine on photosystem II of wheat seedlings under water stress. J. integrative Plant Biol., 45 (8): 920 – 927.

Iordanov, I. and V. Goltsev, 1990. The protective effect of some polyamines on functioning of thylakiod membranes. Fiziologiya na Rasteniyata., 16(4): 42 – 51.

Iqbal, M. and M. Ashraf, 2005. Changes in growth, photosynthesis capacity and ionic relations in spring wheat (*Triticum aestivum* L.) due to pre – sowing seed treatment with polyamines. Plant Growth Regulation, 46: 19 – 30.

Iqbal, M., M. Ashraf, S. Rehman and R. EuiShik, 2006. Does polyamine seed pretreatment modulate growth and levels of some plant growth regulators in hexaploid wheat (*Triticum aestivum* L.) plants under salt stress. Botanical Studies, 47(3): 239 – 250.

Kao, C.H., 1994. Endogenous polyamine levels and dark induced senescence of detached corn leaves. Bot. Bull. Acad. Sinica, 35(1): 15 – 18.

Kesba, H.H., 2005. Effect of amino acids foliar application on Meloigyne incognita and biochemical alterations in grape roots. Bull. Fac. Agric. Cairo Univ., 56(3): 617 – 629.

Krinsky, N.I., 1978. Nov. Photosynthetic functions of carotenoids. In: Advanced Plant Physiology. pp. 225. M. B. Wilkins, ed. Longman Scientific & Technical Group. UK.

Krishnamurthy, R., 1991. Amelioration of salinity effect in salt tolerant rice (*Oryza sativa* L.) by foliar application of putrescine., Plant Cell Physiol., 32: 699-703.

Kuznetsov, V.V. and N.I. Shevyakova, 1997. Stress responses of tobacco cells to high temperature and salinity, proline accumulation and phosphoraylation of polypeptides. Physiol. Planta, 100: 320- 326.

Lawlor, D.W., 1989. Photosynthesis: Metabolism, control and Physiology, pp. 217 – 243. Longman Scientific & Technical Group., Pub. London.

Liu, A.R., Y.B. Zhang and N. Ling, 2002. Effects of spermine and spermidine on several physiological indexes of *Brassica campestris*. Plant Physiol. Comm. Chinese Academy Agric. Sci. (CAAS), Scientech Documentation and Information Center, Beijing, China, 38(4): 349 – 351.

Mansour, M.M.F. and M.M. Al – Mutawa, 1999. Stabilization of plasma membrane by polyamines against salt stress. Cytobios, 100: 7 – 17.

Mansour, M.M.F., Al – M.M. Mutawa, K.H.A. Salama, A.M.F.A. Hadid, R. Ahmed (ed.) and K. A. Malik, 2002. Salt acclimation of wheat salt sensitive cultivar by polyamines. Prospects for Saline Agric., 155 – 160.

Mehta, H.S., R.A. Saftner, R.A. Mehta and P.J. Davies, 1994. Identification of postranscriptionally modified 18-kilodalton protein from rice as eukaryotic translocation initiation factor 5A. Plant Physiol., 106: 1413-1419.

Metzner, H., H. Rau and H. Senger, 1965. Untersuchungen zur synchronisier barkeit ein zelner pigment Mangol Mutanten von chlorella. Planta, 65: 186.

Mizrahi, Y., P.B. Apple White and A.W. Galston, 1989. Polyamine binding to proteins in oat and petunia protoplasts. Plant Physiol., 91: 738-743.

Morse, M.L. and C.F. Carter, 1949. The synthesis of nucleic acid in cultures of *Escherchia coli*, strain B and B/R. J. Bacteriol., 58: 317.

Muller, P. and W. Hilgenberg, 1986. Isomers of zeatin and zeatin riboside in club root tissue: evidence for trans–zeatin biosynthesis by *Plasmodiophora brassicae*. Physiol. Plant, 66: 245–250.

Muting, D. and E. Kaiser, 1963. Spectrophotometric method of determining of amino-N in biological materials by means of the ninhydrin reaction. Seyler's Zschr. Physiol. Chem., 332: 276.

Nag, S., K. Saha and M.A. Choudhuri, 2001. Role of auxins and polyamines in adventitious root formation in relation to changes in compounds involved in rooting .J. Plant Growth Regul., 20: 182- 194.

Nagarajan, S. and J. Rane, 2002. Physiological traits associated with yield performance of spring wheat (Triticum aestivum) under late sown condition. Indian J. Agric. Sci., 72: 135 – 140.

Nassar, A.H., 1997. Physiological responses to polyamines treatments in *Pisum sativum* L. Ph.D. Thesis Faculty of Science Ain Shams Univ. Cairo Egypt.

Nassar, A.H., K.A. El – Tarabily and K. Sivasithamparam, 2003. Growth promotion of bean (*Phaseolus vulgaris* L.) by a polyamine – producing isolate of *Streptomyces griseoluteus*. Plant Growth Regul. Kluwer Academic Publishers, Dordrecht, Netherlands, 40(2): 97 – 106.

Ohlund, J. and T. Nasholm, 2001. Growth of conifer seedlings on organic and inorganic nitrogen sourses. Tree Physiol., 21(18): 1319 – 1326.

Pandy, S., S.A. Ranade, P.K. Nagar and V. Kumar, 2000. Role of polyamines and ethylene as modulators of plant senescence. J. Bio. Sci., 25(3): 291-299.

Papadakis, A.K. and A.K. Roubelakis – Angelakis, 2005. Polyamines inhibit NADH oxidase – mediated superoxide generation and putrescine prevents programmed cell death induced by polyamine oxidase – generated hydrogen peroxide. Planta, 220: 826 – 837.

Paschalidis, A.K. and A.K. Roubelakis – Angelakis, 2005. Sites and regulation of polyamine catabolism in the tobacco plant. Correlation with cell division / expansion, cell cycle progression and vascular development. Plant Physiol., 138: 2174-2184.

Pirie, F.G., 1955. Proteins. In Modern Methods of Plant Analysis. Edited by (Peach K. and Tracey, M. V.). IV: 23-68 Springer Verlag, Berlin.

Pohjanpelto, P. and E. Holtta, 1996. Phosphorylation of Okazaki-like DNA fragments in mammalian cells and role of polyamines in the processing of this DNA. EMBO J., 15: 1193-1200.

Pritsa, T.S. and D.G. Voyiatzis, 2004. Seasonal changes in polyamine content of vegetative and reproduction olive organs and fruit development. Aust. J. Agric. Res., 55(10): 1039-1046.

Reuveni, R,, M. Shimoni, Z. Karchi and J. Kuc, 1992. Peroxidase activity as a biochemical marker for resistance of muskmelon on (*Cucumis meb*) to *Pseudoperonospora cubensis*. Phyto Pathol., 82: 749 – 753.

Rugini, E., A. Jacoboni and M. Luppino, 1993. Role of basal shoot darkening and exogenous putrescine treatments on *in vitro* rooting and on endogenous polyamine changes in difficult to root woody species. Scientia Horti, 53:(1-2) 63 – 72.

Santa – Cruz, A., F. Perez – Aflocea, M. Caro and M. Ascota, 1998. Polyamines as short – term salt tolerance traits in tomato. Plant Sci., Limerick, 138:(1) 9 – 16.

Schmidt, G. and S.J. Thannhauser, 1945. A method for the determination of deoxyribonucleic acid, ribonucleic acid and phosphoproteins in animal tissues. J. Biol. Chem., 161:83.

Sharma, M., B. Kumar and D.M. Pandey, 1997. Effect of pre – flowering foliar application of putrescine on ion composition of seeds of chick pea (*Cicer arietinum* L. cv. H – 82 – 2) raised under saline conditions. Ann. Agri. Bio. Res., 2(2): 111 – 113.

Sheri, L.H., E.S. Ncolas, T.K. Michae and B.G. Joanna, 2000. Comparison of protein expressed by *Pseudomonas aeruginosa* strains representing initial and chronic isolates from a cystic fibrosis patient: an analysis by 2 – D gel electrophoresis and capillary coloumn liquid chromatograph tandem mass spectrometry. Microbiology, 146: 2495 – 2508.

Shindy, W.W. and O. Smith, 1975. Identification of plant hormones from cotton ovules. Plant Physiol., 55: 550–554.

Smith, M.A. and E.J. Wood, 1992. Molecular and Cell Biochemistry Biosynthesis, pp: 128 –134. Chapman and Hell Pubs. London, UK.

Smith, T.A., 1985. Polyamines. Ann. Rev. Plant Physiol., 36: 117 - 143.

Sood, S. and P.K Nagar, 2003. The effect of polyamines on leaf senescence in two diverse rose species. Plant Growth Regul. Kluwer Academic Publishers, Dordrecht, Netherlands, 39: 2 155 – 160.

Suliman, S., C. Wilson and C.M. Grieve, 2002. Effect of salinity and exogenously applied polyamines on growth and ion relations in spinach. J. Plant Nut., 25(12): 2705 – 2717.

Tang, W., R.J. Newton and V. Outhavong, 2004. Exogenously added polyamines recover browning tissues into normal callus cultivars and improve plant regeneration in pine. Physiol. Plant, 122(3): 386-395.

Tassoni, A., F. Antognoni and N. Bagni, 1996. Polyamine binding to plasma membrane vesicles isolated from zucchini hypocotyls. Plant Physiol., 110(3): 817-824.

Theiss, C., P. Bohley and J. Voigt, 2002. Regulation by polyamines of ornithine decarboxylase activity and cell division in the unicellular green alga *Clamedomonas reinhardii.* Plant Physiol., 128: 1470 – 1479.

Tood, C.D. and D.J. Gifford, 2003. Loblolly pine arginase responds to arginine *in vitro*. Planta, 217: (4) 610 – 615.

Uperti, K.K. and G.S.R. Murti, 1999. Effect of polyamines on the changes of endogenous hormones in pea under water stress conditions. Indian J. Plant Physiol., 4: 1-5.

Vervaeke, I., L. Sitichelbout, E. Londers, R. Deroose and M.P. De Proft, 2005. Influence of arginine, ornithine, DFMO and polyamines on division of the generative nucleus in cultured pollen tubes of *Aechmea fasciata* (Bromeliaceae). Plant Cell Tiss. Organ. Cult., 81: 77 – 82.

Vogel, A.J., 1975. A Textbook of Practical Organic Chemistry 3rd ed., English Language Book Society and Longmans Growth Ltd.

Walton, D.C., 1988. Abscisic Acid Biosynthesis and Metabolism. In: Plant Hormones and their Role in Plant Growth and Development, pp: 113 – 131. P.J. Davies, ed. Kluwer Academic Pub. Dordrecht, the Netherlands.

Wasfy, W.S. and E.S. Orrin, 1975. Identification of plant hormones from cotton ovules. Plant Physiol., 55: 550 – 554.

Xie, Z., D. Jiag, T. Dai, Q. Jing and W. Cao, 2004. Effects of exogenous ABA and cytokinin on leaf photosynthesis and grain protein accumulation in wheat ears cultured in vitro. Plant Growth Regul., 44: 25 – 32.

Yemm, E.W. and A.J. Willis, 1954. The respiration of barley plants.IX. The metabolism of roots during assimilation of nitrogen. New Phytol., 55: 229 - 234.