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Growth and physiological responses of *Solanum lycopersicum* to atonik and benzyl adenine under vernalized conditions

Samia Ali Haroun, Wafaa Mohamed Shukry*, Mohamed Ali Abbas and Amr Mohamed Mowafy

Department of Botany, Faculty of Science, Mansoura University, Egypt.

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A foliar application of Atonik (250, 500 and 1000 ppm) and benzyl adenine (25, 50 and 100 ppm) under vernalization was investigated on *Solanum lycopersicum* (var. Beto 86) plant. All determined growth parameters (root length, root fresh and weights, shoot length, number of leaves, number of nodes, total leaf area, shoot fresh and dry weights and relative water content) were inhibited in response to treatment with vernalization. Meanwhile vernalization in combination with varying concentration of Atonik or benzyl adenine (BA) led to a general significant increase in these parameters. Vernalization alone or in combination with Atonik or benzyl adenine accelerated flowering especially in response to 1000 ppm Atonik or 50 ppm BA under vernalization treatment. Vernalization treatment significantly increased the concentration of chlorophyll *a* and *b*, carotenoids and consequently total pigments. While chlorophyll *a/b* is insignificantly affected. In general, vernalization and different concentrations of Atonik or BA led to a massive increase in these pigments. Glucose, sucrose, polysaccharides, ammonia, amino and soluble as well as total nitrogen and protein were increased in tomato plants under the influence of vernalization alone or in combination with Atonik or BA. Moreover, a general significant increase in the content of K^+, Na^+ and Ca^{++}, were detected in both tomato root and shoot as a result of treatments. Vernalization caused a significant decrease in total auxins, gibberellic acid and different cytokinin fractions in the shoot of tomato plants, whereas abscisic acid increased significantly by this treatment. At vernalization, all concentrations of Atonik or BA reverse this situation as compared with control values.

Key words: Atonik, benzyl adenine, *Solanum lycopersicum*, vernalization.

INTRODUCTION

Vernalization is the process by which prolonged exposure to cold temperatures promotes flowering. Over the past century, this process has been studied extensively at the physiological level (Amasino, 2004). A great deal of attention has been focused upon the application of several plant growth regulators in order to improve qualitatively and/or quantitatively the yield of many crop plants. Positive or negative responses could be achieved depending on the plant species and type of growth regulator employed (Makunga et al., 2003). Hwan et al. (2010) stated that vernalization effects flowering in *Arabidopsis thaliana* plants upon exposure to inductive photoperiods in winter annuals and biennials. Sanders and Cure (1996) observed that in 'Buffalo' and 'Granex 33' onions, an increase in vernalization period resulted in a decrease in the number of the days required for flowering and also led to reduction in percentage flowering relative to control. Overall dry matter accumulation was unaffected but fresh mass per bulb decreased with increased vernalization days. The response to vernalization involves two phases. The first is a cold perception that measures the cumulative time of

*Corresponding author. E-mail: wafaa_12@yahoo.com

exposure to cold. The second phase is essentially the output of the cold perception. When a sufficient duration of cold has been perceived, a series of changes of gene expression ensue, ultimately leading to the epigenetic repression of Flowering Locus *C* (*FLC*). VIN3, which is a repressive chromatin-remodeling component, is induced only after a sufficient duration of cold shoke treatment. One of the early molecular events in the vernalization response is the induction of VIN3 by prolonged cold exposure. Upstream of VIN3, there must be a biochemical mechanism to sense cold. However, nothing is known about the upstream event.

The induction of VIN3 by cold is unique in that VIN3 induction takes several days of cold, unlike many cold-induced genes, which are induced within hours of cold exposure (Thomashow, 2001). Furthermore, VIN3 mRNA expression is quickly repressed once plants are moved to warm temperature.

Stapleton and Jones (1987) found that vernalization treatment of *Lolium perenne* cv. Vigor increased expanding and area of the leaf significantly and consequently increased the photosynthetic capacity. Galiba et al. (1997) found that, the major changes in osmotic potential during cold treatment are due to change in sugar concentration and concluded that, there is a correlation between sugar content and frost tolerance. During the period of vernalization, the protein content in the cell increased in comparison with the nucleic acid while the protein content decreased as a proportion to dry matter (Nichita and Dascalivc, 1994). Taeb et al. (1992) recorded less sodium accumulation when dominant alleles of vernalization or photoperiod requirement are present in the same family (Triticeae). Tsybul and Karpukhina (1991) observed that the activity of auxins and cytokinins in wheat was higher in vernalized winter and spring plants than in slowly developing unvernalized ones. Also, it was suggested that vernalization influences gibberellic acid (GA3) content and GA3 metabolism, with GAs serving as probable regulatory intermediates between vernalization and subsequent stem growth (Zanewish and Rood, 1995). II'– Yashuk and Likholat (1989) remarked that exposing winter wheat plants, at the 3 leaf stage, to 3°C for 2 h (led to an) increase in the abscisic acid (ABA) content in the shoots while (Mi and Li, 1995) stated that, the concentrations of GA_3, ABA and the ratio of GA_3: ABA were not significantly affected by vernalization in the embryos of spring wheat.

Atonik is a relatively new growth substance composed of 3 aromatic nitro compounds (Sodium 5-nitroguaiacolate, sodium 1-nitrophenolate and sodium 4-nitrophenolate) that enhances growth and some essential metabolic processes of treated plants (Guo and Oosterhuis, 1995).

Data concerning the effectiveness of Atonik in crop improvement are different and depend on plant species and sometimes on variety. Arora et al. (1981) reported that, the spraying of Atonik at concentration 0.05% on tomato at flowering stage or at fruit set gave about a 37% increase in crop yield and an increase for 29% if it was applied at 0.15% (Sumiati ,1989).

Tomato sprayed twice with Atonik at the beginning of flowering and beginning of fruit set on the first truss increased the number of fruits by about 24% and crop by 20% (Shi and Shi, 1999). Atonik at concentration 0.08% used 3 times at weekly intervals, before flowering of hot pepper (*Capsicum annuum*), increased yield about 13% (Srinivas et al., 1986). When used for foliar spray of sugarbeet, it increased yield by 3 to 5% (Zahradnicek and Pulkrabek, 2001). When its compounds were applied at concentration 0.05 to 0.1% for spraying apple cultivars in the 10 days after petals fall, they did not affect the fruit yield but increased the number of fruits more than 65 mm in diameter by 12 to 16% (Koupil, 1997). Atonik, applied immediately after harvest, increased the yield of strawberry cultivars by 3 to 30% in the following year (Eftimov, 1988). On the other hand Castro et al. (1987) found an inhibition in growth of radicale and hypocotyls when seeds of tomato cv.Kada treated with 0.5 ml/L of Atonik. Treatment of sugarbeet (*Beta vulgaris* L.) with Atonik increased root yield by an average 3.4% over 3 years, while sugar yield was 4.3% more than the control variety (Pulkrabek, 1996). Lee et al. (1990) stated that in sweet potato, starch content and yield increased in plants from cuttings treated with 1000 or 2000 ppm Atonik. Also Azab et al. (1993) found that, cotton cv.Giza 80 which sprayed twice with 1, 2 or 3 ml/L. Atonik showed increased seed yield but did not cause an increase in fiber quality or seed oil or protein contents.

Benzyl adenine (BA) is one of the synthetic cytokinins which plays permissive role in the regulation of various growth processes in the plants (Skoog et al., 1967 and Ibrahim et al., 2010). Shibli et al. (2001) found that, BA up to 1.0, 1.5 and 2.0 mg/L, led to an increase in number of proliferated shoots and number of nodes per flask *in vitro* culturing of *Solanum tuberosum* cv 'Spunta' shoot on liquid media containing BA . Nielsen and Ulveskov (1992) recorded the stimulation of leaf expansion by application of exogenous cytokinins on *Capsicum* leaf discs. The effect of cytokinins especially benzyl adenine on the plant growth and chemical constituents of different species are mentioned by Eraki et al. (1993) on *Salvia officinalis* plants, Mazrou (1992) on *Datura*, Mazrou et al. (1994) on sweet basil and Vijakumari (2003) on *Andrographis panculata*. Chernyad– ev (2000) found that 50 mg/L Benzyle adenine (BA) enhanced the area, weight and specific weight of the expanding leaf as well as the content of chlorophylls, carotenoids, proteins, and activity of rubisco in sugarbeet. Also (Zhang et al. 1997) found that BA (50 µmole/L) increased dry weight of plants and chlorophyll concentration in leaves of wheat (*Triticum aestivum*). Ni et al. (2000) sprayed ponkan (Citrus reticulata) with BA and found that BA increased the contents of total soluble sugars, reducing sugars and vitamin C, but decreased the content of fruit acids. Thus

the fruit quality of ponkan was improved by BA treatment. Feito et al. (1994) studied the endogenous cytokinins and BA metabolites in kiwi fruit explants grown on solid field and liquid media supplemented with BA and found that the content of cytokinins is influenced by the level of BA. This result suggested that phytohormones in increased total uptake of N^{-}, P^{-3}, K^{+}, Na^{+}, Ca^{+2} and Mg^{+2} (Wyszkowska, 1999). Meanwhile Saleh et al. (2002) studied the effect of spraying with BA at 50 mg kg^{-1} on mineral status in barley (*Hordeum vulgare*) plants and grains and found that, the concentration of P, K, Ca, Na, and Fe have not been affected.

MATERIALS AND METHODS

The effect of Atonik (250, 500 and 1000 mg/L) and BA (5, 50 and 100 mg/L) on growth criteria (root length, root fresh and dry weights, (shoot length, shoot fresh and dry weights, number of nodes, number of leaves, total leaf area and relative water contents) and metabolic activities, as well as photosynthetic pigments, carbohydrates content, nitrogen fractions, total nitrogen and protein, were determined. In addition, ion content (K^{+}, Na^{+} and Ca^{2+}), as well as plant growth regulators (PGRs) (auxin, GAs, ABA and CKs) were determined in tomato plants (*S. lycopersicum* (var.Beto 86) family Solanaceae. Atonik and BA were purchased from Sigma-Aldrich, St. Louis, MI, USA.

Time course of experiment

Homogenously-sized seeds of *S. lycopersicum* were supplied by the Agricultural Research Center, Ministry of Agriculture, Giza, Egypt. Seeds were selected and their surface sterilized with 0.01% $HgCl_2$ solution then washed thoroughly with distilled water. The seeds were then divided into equal groups of 30 seeds each. Each group was placed in a plastic box (21 × 14 cm) containing filter paper saturated with water. Boxes were incubated at 5°C for 5 days (after carrying out preliminary experiments at different temperatures in which 5°C was shown to be the lowest temperature at which germination occurred). This constituted the vernalization treatment. After vernalization, boxes were incubated at room temperature for 12 h before sowing. Other separate sterilized groups were placed in a plastic box contained filter paper saturated with water and were incubated at room temperature for 12 h before sowing, these served as the control. All seeds were cultivated on the 3rd of February in pots (30 cm in diameter) containing equal amounts of homogenously mixed soil (sand: clay, 1: 2, v/v). Ten seeds were sown and irrigated, when required, by adding equal amounts of water to each pot. All plants were exposed to normal day length and natural illumination. Super phosphate and urea fertilizers were added to the soil during the first week of cultivation. Sampling from control and vernalized plants took place 77 days after sowing (DAS) at 21st of April (initial stage). After initial sampling, and in the same day, the pots containing vernalized plants were divided into 7 treatments, including the control. The first treatment was sprayed with water and considered as the vernalization treatment. The remaining treatments were sprayed separately with BA at 25, 50, 100 mg/L, and with Atonik at 250, 500 and 1000 mg/L. At the 7th of May (93 DAS) and the 7th of June (123 DAS) sampling of the plants takes place (stage I and stage II) which corresponding to vegetative stage and flowering stages, respectively. The samples collected at different stages were used to assess growth parameters as well as different metabolic activities (pigments, carbohydrate fractions and N fractions). In addition, the content of ions and PGRs were also determined. All chemicals used in this study were purchased from Sigm-Aldrich, St. Louis, MI, USA.

Analytical methods

Estimation of the relative water contents

Based on the method described by Pardossi et al. (1992), leaf samples from the third node were immediately sliced into 2 cm sections, weighed to obtain their fresh weight (FW), then floated on distilled water at 20°C in dim light for 4 h. After this period, turgid leaf slices were rapidly and gently blotted and weighed to obtain the turgid weight (TW). Leaf slices were then dried at 70°C until the constant dry weight (DW) was obtained. Leaf relative water content was calculated by the following formula:

$$\text{Relative water content \%} = (FW - DW)/(TW - DW) \times 100$$

Estimation of photosynthetic pigments

The plant photosynthetic pigments (chlorophyll (Chl) *a*, Chl *b* and carotenoids) were determined at different stages of plant growth using a spectrophotometric method as recommended by Arnon (1949) for Chl and Horvath et al. (1972) for carotenoids as adopted by Kissimon (1999). Chlorophyll concentration was calculated as µg/g DW of leaves.

Estimation of carbohydrates

The methods adopted in this investigation for extraction of the different carbohydrate fractions tested were essentially those described by Yemm and Willis (1954) and Handel (1968). Dry tissue samples were submerged overnight in 10 ml 80% (v/v) ethanol at 25°C with periodic shaking. The ethanolic mixture was filtered and the ethanolic filtrate was made up to a certain volume and kept in the refrigerator for analysis of the different sugar fractions. Glucose, according to Fetris (1965) as modified by Riazi et al. (1985), Sucrose, according to Handel (1968) and polysaccharides, according to Thyermanavan and Sadasivam (1984), were estimated in the extract.

Estimation of nitrogenous constituents

The method used in this study was essentially that adopted by Yemm and Willis (1956). Ammonia-N was estimated spectrophotometrically by the method of Delory (1949) using Nessler's reagent as modified by Naguib (1964). The method used for estimation of amide-N was that recommended by Naguib (1964). Amino-N was estimated according to Muting and Kaiser (1963). The total soluble nitrogen and total nitrogen were determined by the conventional semi micro modification of Kjeldahl method Pirie (1955) and Rees and Williams (1943) respectively.

Estimation of ions

Flame photometer was used for determining potassium and sodium, while calcium and magnesium were measured by atomic absorption spectrophotometer according to the method described by Chapman and Pratt (1978).

Estimation of plant growth regulators (PGRs)

Extraction and separation: Extraction, separation were performed

according to Shindy and Smith (1975). Methylation of plant hormones diazomethane was obtained from methylamine hydrochloride as reported by Vogel (1975) as follows: Methylamine solution (100 g) was placed in a 500 ml flask and concentrated hydrochloric acid (78 ml) was added. Water was added to bring the total weight to 250 g, then urea (150 g) was introduced and the mixture was boiled gently under reflux for 2 min and then vigorously for 15 min. The solution was cooled to room temperature, then sodium nitrate (55 g) was dissolved in it and a mixture of 300 g of crushed ice and 50 g concentrated sulfuric acid was prepared in a 1500 ml beaker surrounded by an ice bath and salt. Cold methyl urea-nitrate solution was added slowly with mechanical stirring at such a rate that the temperature did not rise above 0°C.

The crystalline nitrosomethyl urea was filtered at once then drained well and dried in a vacuum desiccator. An aqueous potassium hydroxide solution (50 ml, 50%) and ether (200 ml) were placed in a 500 ml round-bottomed flask. The mixture was cooled to 5°C. Nitrosomethyl urea (20.56 g) and ether (80 ml) were then added. The ethereal layer was separated using a separating funnel and dried over pellets of potassium hydroxide for 2 to 3 h.

Methylation with diazomethane

PGRs fractions and standard of PGRs were dissolved in a little anhydrous ether and the ethereal solution of diazomethane was added in small portions until gas evolution ceased. The mixture acquired a pale yellow colour indicated the addition of excess diomethane. The reaction mixture was left for 10 min and ether was distilled off using a hot water bath. The residue was dissolved in a minimal amount of acetone and used for gas-liquid-chromatography (GLC) analysis using a Hewellet Packard (HP-GC-MS model).

Separation of methyl esters of plant hormones by gas liquid chromatography

Analysis of methyl esters of organic acids was performed on a Pye Unican GCV gas chromatograph equipped with a dual flame ionization detector. The gas liquid chromatographic conditions for isothermal work were as follows: 2.8 m × 4 mm glass column packed with acid, alkali and silanized diatomite C (100 to 120 mesh) and coated with 1% OV-17, temperature, injector 250°C, column 230°C, and detector 300°C flow rates, nitrogen 300 ml/min, hydrogen 33 ml/min, and air 330 ml/min range of 32 × 102 and chart speed of 3 cm/min.

Identification and determination of auxins, GAs and ABA

Peak identification was performed by comparison of the relative retention time for each with those of standard PGRs (IAA, GA and ABA) from Sigma-Aldrich (Shindy and Smith, 975).

Identification and determination of CKs

Aqueous fractions were combined and then adjusted to pH 5.5 by 1% NaOH and partitioned 3 times with 50 to 100 ml of water-saturated *n*-butanol. Then *n*-butanol fractions were combined and reduced in volume to 5 ml, and stored at -20°C until GLC analysis for CKs (Horgan et al., 1973).

Statistical analyses

The results were statistically analyzed and comparison among means (three samples) was carried out using Statgrapfic – vers-4-2-Display (one - tailed ANOVA) as described by Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

The estimated growth parameters (root length, root fresh weight, root dry weight, shoot length, number of nodes, number of leaves, total leaf area, shoot fresh and dry weights and relative water content) decreased significantly, in general, during the different growth stages of tomato when subjected to vernalization treatment, as compared to control (Table 1). In this respect, Chauvel et al. (2002) stated that, three populations of *Alopecurus myosroides* were affected by vernalization via a decrease in biomass by reducing tiller number. Filek et al. (2000) found that, accumulation of dry weight was higher in vernalized than non-vernalized *Vicia faba* L. minor plants. On the other hand, Stapleton and Jones (1987) found that the leaf area of perennial ryegrass (*Lolium perenne* L.) increased under vernalization treatment, compared to the control value. A general significant increase was detected during tomato growth in the previous growth parameters in response to treatment with the used concentrations of either Atonik or BA under vernalization condition (Table 1). The magnitude of increase seemed to be more pronounced in case of treatment with 1000 ppm Atonik or 50 ppm BA. In this connection, (Sumiati and Herbagiandono, 1987) found that, Atonik treatment increased fresh and dry weights of *Pleurotus ostreatus*. Also, Gomaa and Stino (1989) observed that, Atonik increased the number of internodes of apple hence shoot length was increased. Moreover, Park et al. (1998) found, at flowering of balloon flower (*Platycodon grandiflorus*) that, fresh and dry mass and leaf area increased with decreasing temperature. On the other hand, Castro et al. (1987) found that, Atonik inhibits radicale and hypocotyls growth in tomato plant. In addition, (Abdel-Al and Ismail, 1990) found that, Atonik application reduces cotton plant height. Concerning the effect of BA, Kang et al. (1997) observed that, BA increased number of shoots but decrease shoot length of *Satsuma mandarin*. Also, in sugarbeet plant, Chernyad-ev (2000) found that, BA treatment increased area and specific weight of the expanded leaf. Moreover, BA treatment favored shoot differentiation and had an inductive effect on bud formation of *Anthurium andraeanum* Cen et al. (1993) and apple (Wertheim and Estabrooks, 1994). In the present study, it can be concluded that the reduction in growth parameters in response to vernalization treatment may be related to the changes in the endogenous growth hormones. In this respect, Tsybul and Karpukhina (1991) concluded that reduction in growth of wheat may be explained by variation in the level of the endogenous hormones by vernalization which led to changes in the rate of growth and development. On the other hand, the stimulation of tomato growth as a consequence of Atonik or BA treatments may be due to the excessive uptake of

Table 1. Effect of vernalization and different concentrations of Atonik or benzyladenine on growth parameters of *Solanum lycopersicum* plant.

Stage	Treatment	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Shoot length (cm)	Number of nodes/ plant	Number of leaves/ plant	Leaf area/ plant (cm^2)	Shoot fresh weight (g)	Shoot dry weight (g)	relative water content
	Control	4.866	0.132	0.021	4.933	3.198	2.56	10.791	0.475	0.066	89.233
Initial	Vernalized	4.183*	0.093*	0.014*	4.266*	2.916*	1.933*	8.246*	0.406*	0.043*	83.003*
	LSD	0.229	0.013	0.005	0.279	0.105	0.129	0.923	0.047	0.005	3.423
	Control	5.71	0.182	0.033	5.596	3.784	3.39	34.993	0.654	0.116	90.966
	Vernalized	5.238*	0.134*	0.027*	5.07*	3.256*	2.98*	28.443*	0.535*	0.09*	83.39*
	Vernalized+Atonik 250	6.168*	0.245*	0.037	5.88	4.14*	3.792*	38.843*	0.733*	0.13*	93.54
	Vernalized+Atonik 500	6.561*	0.285*	0.046*	6.713*	4.606*	4.473*	47.343*	0.843*	0.141*	95.916*
Stage I	Vernalized+ Atonik 1000	6.931*	0.373*	0.069*	8.636*	5.208*	4.78*	50.533*	0.986*	0.15*	96.53*
	Vernalized+BA 25	6.143*	0.239*	0.053*	5.746	4.009	3.591	38.166*	0.726*	0.135*	92.58
	Vernalized+BA 50	6.676*	0.269*	0.063*	6.753*	4.821*	4.506*	48.316*	0.801*	0.144*	93.713
	Vernalized+BA 100	5.403	0.17	0.037	5.496	3.82	3.43	38.36*	0.604	0.106*	87.153*
	LSD	0.333	0.024	0.004	0.343	0.268	0.318	1.779	0.052	0.004	2.503
	Control	12.79	0.373	0.059	12.433	5.214	4.29	100.968	1.265	0.371	88.67
	Vernalized	11.823*	0.301*	0.049*	11.46*	4.908	3.506*	80.833*	1.137*	0.243*	86.36
	Vernalized+Atonik 250	14.003*	0.461*	0.064	13.823*	5.474	5.056*	114.566*	1.415*	0.425*	92*
	Vernalized+Atonik 500	15.2*	0.5*	0.071*	15.473*	6.664*	5.276*	121.666*	1.494*	0.526*	94.28*
StageII	Vernalized+ Atonik 1000	16.756*	0.63*	0.084*	17.62*	8.8*	6.443*	131.333*	1.935*	0.826*	96.333*
	Vernalized+BA 25	14.183*	0.478*	0.068*	14.006*	6.28*	4.71	108.266*	1.37	0.459*	90.2
	Vernalized+BA 50	14.843*	0.505*	0.081*	16.6*	7.523*	5.29*	122.666*	1.67*	0.563*	94.173*
	Vernalized+BA 100	12.49	0.331	0.055	12.02	5.951*	4.013	98.046	1.163	0.306*	90.176
	LSD	0.823	0.044	0.006	0.79	0.474	0.477	5.016	0.101	0.02	2.482

(*) = Significant increase or decrease at 0.05 LS.

uptake of minerals and nutrients from soil and/ or the level of the endogenous hormones (Tables 6 and 7). In harmony, Pulkrabek (1996) attributed the greater effect of Atonik or BA on the number and quality of harvested sugarbeet, to the positive effect on the number of vascular bundles (+ 2% against untreated control). Furthermore, Mohsen and Zaki (1998) remarked a strong relation between vernalization and the level of the endogenous hormones in *Triticum aestivum* plants.

Changes in flowering response

The present results indicate that, the treated plants with vernalization alone or in combination with Atonik or BA, appeared to interrupt juvenility and to cross the threshold to flowering, whereas, the juvenile stage was still prevailing in the corresponding control plants. Thus, the start of flowering was earlier by one day in the vernalized tomato plants. Moreover, it was found that, induction of flowering was earlier by 3, 4 and 6 days in tomato plant by the 250, 500 and 1000 ppm Atonik respectively. Also the start of flowering was earlier by 2, 5 and 1 days in response to tomato treatment with BA at 25, 50 and 100 respectively.

The induction of flowering in tomato plants treated with vernalization either alone or in combination with increasing concentrations of Atonik or BA may suggest that, under, these

Table 2. Effect of vernalization and different concentrations of Atonik or benzyl adenine on date of flowering and number of flower/plant of *Solanum lycopersicum* plant during May 2002.

Treatment	Date of flowering	Number of flowers/ plant
Control	7 /5/ 2002	1.89
Vernalized	6 /5/ 2002	2.23
Vernalized + Atonik 250	4 /5/ 2002	2.4
Vernalized + Atonik 500	3 /5/ 2002	3.1
Vernalized + Atonik 1000	1 /5/ 2002	3.9
Vernalized + BA 25	5 /5/ 2002	2.12
Vernalized + BA 50	2 /5/ 2002	2.8
Vernalized + BA 100	6 /5/ 2002	2

treatments play different roles according to plant life cycle and the type of treatment and its concentrations (Table 2). In consistence with our results, Sheldon et al. (1999) reported an induction of flowering in *Arabidopsis* by vernalization treatment.

The same findings were obtained by Hemming et al. (2008) and Karsai et al. (2008) in barley (*Hordeum vulgare)*. Also Kolodziej (2001) found that, Atonik treatment increase yield of *Lagenaria siceraria*. Moreover, Rylott and Smith (1990) stated that the application of cytokinins to flowers caused an active cell division of the embryo, and hence the attraction of assimilates to the new developing pods from other plant parts. Ramanayake et al. (2001) demonstrated that BA when applied to the giant bamboo (*Bambusa oldhamii*) it caused shortening of the time required for plants to initiate first floral buds.

Changes in photosynthetic pigments

At all stages of *S. lycopersicum* growth, the determined photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophylls, carotenoids and total pigments) significantly increased under vernalization treatment as shown in Table 3. These results are in agreement with Stapleton and Jones (1987) who found an increase in photosynthetic capacity of *Lolium perenne* under vernalization treatment. Also, (Roberts et al., 1993) detected an increase in chlorophyll synthesis in wheat under cold treatment. Vernalization in combination with the used concentrations of Atonik or BA led to additional increases in the investigated pigments in relation to control value. On the other hand, chlorophyll a/ b ratio seemed to be non-significantly affected in response to the aforementioned treatments. These results are with those obtained by Zayad et al. (1985) on rosette plants (*Agave americana)*, Rani et al. (1988) on mung bean *(Vigna radiate*), Oosterhuis and Egilla (1996) on cotton plants. In this connection, Petrenko and Biryukova (1977) suggested that, kinetin may cause an increase in the concentration of carotenoids which may protect chlorophyll against degradation by photooxidation.

Changes in carbohydrates

In relation to control values, glucose, sucrose and polysaccharides contents, in general, significantly increased during different growth stages of tomato grown under the vernalization condition (Table 4). In support, Lexander and Atherton (1987) found that, glucose, fructose and sucrose concentration increased in *Vicia faba* L. minor plants grown at 10°C. In addition vernalization with various concentrations of Atonik or BA generally caused a significant increase in all estimated carbohydrate fractions. In this respect, Erwin et al. (2002) suggest that, the gene regulating sucrose accumulation in Japanese radish *(Raphanus sativus* var. *longipinnatus)* was closely associated with the effect of vernalization. Ni et al. (2000) stated that, BA increase reducing sugars and total soluble sugars in ponkan (*Citrus reticulata*).

The stimulation of the content of carbohydrate in tomato plants, treated with Atonik or BA under the effect of vernalization, appear to coincide with the stimulated changes in leaf area, dry weight and photosynthetic pigments as well as the content of growth stimulators (Tables 1, 3, 7). In this connection, Mohsen and Zaki (1998) studied the effect of BA on the growth and metabolic activities of *T. aestivum* seedlings and detect variable positive relationships between sugars, carbohydrates and concentration of chlorophyll and carotenoids.

Changes in nitrogen fractions

Perusal of data presented in Table 5 revealed that, remarkable increases in ammonia, amino nitrogen, total soluble nitrogen, protein and consequently total nitrogen were observed as a result of treatment tomato plants with vernalization alone or in combination with different

Table 3. Effect of vernalization and different concentrations of Atonik or benzyl adenine on photosynthetic pigments content, in mg/g dry weight of *Solanum lycopersicum* plant.

Stage	Treatment	Chlorophyll a	Chlorophyll b	Chlorophyll a + Chlorophyll b	Chlorophyll a/ Chlorophyll b	Carotenoids	Total pigment
Initial	Control	0.0596	0.0273	0.0869	2.1863	0.016	0.1029
	Vernalized	0.0731*	0.0373*	0.1105*	1.9623*	0.0208*	0.1313*
	LSD	0.0038	0.0018	0.0034	0.211	0.0007	0.004
Stage I	Control	0.0684	0.0353	0.1037	1.9376	0.0228	0.1266
	Vernalized	0.0811*	0.0431*	0.1242*	1.8849	0.0265*	0.1508*
	Vernalized + Atonik 250	0.095*	0.0483*	0.1434*	1.9717	0.0306*	0.1741*
	Vernalized + Atonik 500	0.105*	0.0533*	0.1583*	1.9718	0.0346*	0.193*
	Vernalized + Atonik 1000	0.1147*	0.0593*	0.1741*	1.9342	0.0404*	0.2145*
	Vernalized + BA 25	0.0941*	0.0464*	0.1406*	2.0303	0.0305*	0.1711*
	Vernalized + BA 50	0.1044*	0.0517*	0.1561*	2.017	0.0382*	0.1944*
	Vernalized + BA 100	0.0863*	0.0443*	0.1307*	1.9444	0.0285*	0.1592*
	LSD	0.0053	0.0024	0.0055	0.122	0.0015	0.0055
Stage II	Control	0.0342	0.0159	0.0502	2.141	0.0182	0.0684
	Vernalized	0.0428*	0.0189*	0.0618*	2.2617	0.0204*	0.0822*
	Vernalized + Atonik 250	0.0477*	0.0212*	0.0689*	2.2546	0.0255*	0.0945*
	Vernalized + Atonik 500	0.0529*	0.0274*	0.0804*	1.9292	0.0305*	0.1109*
	Vernalized + Atonik 1000	0.0631*	0.0331*	0.0963*	1.9053	0.035*	0.1313*
	Vernalized + BA 25	0.0456*	0.0216*	0.0672*	2.1182	0.0221*	0.0894*
	Vernalized + BA 50	0.0571*	0.0256*	0.0827*	2.2314	0.025*	0.1077*
	Vernalized + BA 100	0.0449*	0.0196*	0.0645*	2.2892	0.0219*	0.0864*
	LSD	0.0031	0.0016	0.004	0.2162	0.0016	0.0047

(*) = significant increase or decrease at 0.05 LSD.

concentrations of Atonik or benzyl adenine during different stages of growth and development. In harmony, Streck et al. (2003) found that, cold treatment led to induction of amino acid, polyamines and proline in wheat plant. Moreover, Renaut et al. (2005) found that there was a rapid accumulation of protein in poplar *(Populus tremula*) plantlets under cold conditions. Also, Nowak et al. (1997) observed that, BA increase nitrogen content in seeds, pods and stems and they suggested that, BA application increase uptake of nitrogen in bean (*Phaseolus vulgaris*) plants. Further, Shadi et al. (2001) stated that, BA inhibited biosynthesis of high molecular weight protein and stimulated biosynthesis of mid and low molecular weight protein in some maize inbred. Ramadan (1992) and Mac Millan et al. (2005) detected significant increment in total soluble-N, protein and total-N of broad bean (*Vicia faba*) and *Lolium perenne* respectively under the effect of growth regulators.

Table 4. Effect of vernalization and different concentrations of Atonik or benzyl adenine on carbohydrates content, in mg/g dry weight of *Solanum lycopersicum* plant.

Stage	Treatment	Glucose	Sucrose	Polysaccharides
Initial	Control	0.383	6.473	138.63
	Vernalized	0.402*	6.866*	161.8*
	LSD	0.023	0.228	15.782
Stage I	Control	0.492	8.591	231.573
	Vernalized	0.508	8.962	247.14*
	Vernalized+Atonik 250	0.572*	11.466*	250.84*
	Vernalized+Atonik 500	0.641*	14.22*	292*
	Vernalized+ Atonik 1000	0.741*	17.923*	323.89*
	Vernalized+BA 25	0.523*	9.61	243.04
	Vernalized+BA 50	0.679*	12.573*	265.373*
	Vernalized+BA 100	0.511	9.31	241.333
	LSD	0.024	1.075	14.046
Stage II	Control	0.802	12.78	274.805
	Vernalized	0.852	13.013	281.578
	Vernalized+Atonik 250	0.915	17.833*	281.029
	Vernalized+Atonik 500	1.065*	20.666*	312.333*
	Vernalized+ Atonik 1000	1.319*	25.666*	365.333*
	Vernalized+BA 25	1.059*	16.533*	312.218*
	Vernalized+BA 50	1.14*	20.4*	359.666*
	Vernalized+BA 100	0.936	13.58	285
	LSD	0.16	1.4	18.309

Changes in ions content

Treatment of tomato plants with vernalization led to a significant increase in K^+, Na^+ and Ca^{++} contents in both roots and shoots at different stages of growth as shown in Table 6. In this connection, Pressman et al. (1993) found relatively marked decrease in Ca^{++} at the margins of the vernalized leaves of Chinese cabbage (*Brassica rapa)*. With respect to the effect of vernalization and different concentrations of Atonik or BA, a general significant increase in the content of K^+, Na^+ and Ca^{++} ions were detected in both root and shoot.

The magnitude of increase in element contents was most pronounced with the increase in Atonik concentrations and with 50 ppm BA, as compared to the control value. The results herein are in agreement with Taeb et al. (1992), Zahradnicek et al. (1996) and Ellis et al. (2000). At the same time the observed changes in the inorganic ions contents may be due to the effects of Atonik or BA on the vascular bundles of tomato plants. Thus, Pulkrabek (1996) stated that Atonik had a great effect on the number of vascular bundles (+2% against untreated control).

According to Table 6 it was found that the changes in K^+, Na^+ and Ca^{++} in shoot and root of tomato plants in response to various treatments is expected to be the influence of Atonik or BA on protein synthesis (Table 5). Proteins are required to transport protons, inorganic ions and organic solutes across the plasma membrane and tonoplast at rates sufficient to meet the needs of the cells (Schreoder et al., 1999). Channel proteins facilitate the diffusion of water and ions down energetically favorable gradients. These results are similar with the findings of Rawia and Bedour (2006) on Croton (*Codiaeum variegatum*), where they revealed that the plants sprayed with different concentrations of BA (25, 50 and 75 ppm) increased the concentration of minerals N^-, P^3, K^+, Mg^{+2}, Fe^{+3}, Zn^{+2}, Mn^{+2}, and Cu^{+2} in caroton plants comparing with the control treatment. Moreover, Kuiper et al. (1992) found that BA increase ATPase activity. Taiz and Zeiger (2003) stated that undervernalization treatment, positively charged macronutrients such as potassium (K^+), and calcium (Ca^{++}) are required in relatively large amount for plant growth and development.

Table 5. Effect of vernalization and different concentrations of Atonik or benzyl adenine on different nitrogen fractions, in mg/g dry weight of *Solanum lycopersicum* plant.

Stage	Treatment	Ammonia	Amide Nitrogen	Amino Nitrogen	Total soluble Nitrogen	Total Nitrogen	Protein
Initial	Control	0.797	0.528	0.289	2.473	21.65	19.176
	Vernalized	1.05*	0.307*	0.382*	3.12*	26.8*	23.68*
	LSD	0.028	0.011	0.005	0.136	1.837	1.79
Stage I	Control	1.158	1.046	0.569	3.473	32.416	28.943
	Vernalized	1.293*	0.875*	0.789*	3.97*	36.916*	32.946*
	Vernalized + Atonik 250	1.438*	1.414*	0.922*	4.146*	40.416*	36.27*
	Vernalized + Atonik 500	1.67*	1.23*	1.007*	4.366*	42.675*	38.309*
	Vernalized + Atonik 1000	1.831*	1.115*	1.143*	4.804*	47.261*	42.457*
	Vernalized + BA 25	1.351*	1.387*	0.888*	3.906*	38.3*	34.393*
	Vernalized + BA 50	1.585*	1.131*	1.011*	4.503*	44.2*	39.696*
	Vernalized + BA 100	1.359*	1.089*	0.971*	3.534	37.1*	33.566*
	LSD	0.032	0.015	0.012	0.115	1.285	1.272
Stage II	Control	0.797	0.876	0.49	3.084	27.6	24.515
	Vernalized	0.921*	0.73*	0.562*	3.472*	30.22*	26.747*
	Vernalized + Atonik 250	1.136*	1.173*	0.782*	4.129*	36.45*	32.321*
	Vernalized + Atonik 500	1.258*	1.011*	0.898*	4.239*	37.7*	33.46*
	Vernalized + Atonik 1000	1.453*	0.898*	1.026*	4.53*	40.216*	35.686*
	Vernalized + BA 25	1.033*	1.109*	0.741*	3.625*	33.05*	29.425*
	Vernalized + BA 50	1.166*	1.073*	0.94*	4.141*	37.75*	33.609*
	Vernalized + BA 100	0.993*	0.967*	0.805*	3.49*	31.166*	27.676*
	LSD	0.033	0.015	0.011	0.109	1.072	1.083

(*) = Significant increase or decrease at 0.05 LSD.

Changes in growth regulators

During vegetative growth, the recorded data in Table 7 showed that, vernalization led to a significant decrease in total auxins, gibberellic acid and different cytokinins fractions (zeatin, kinetin and benzyl adenine) in the shoot of tomato plants. In contrast ABA increased significantly by this treatment, as compared to the control level. The aforementioned data are in agreement with Rietveld et al. (2000) who suggested that the essential developmental step of vernalization is to increase the sensitivity to growth regulators IAA.

Moreover, Il-Yashuk and Likholat (1989) observed a high level of ABA at the end of vernalization of wheat plants. On the other hand, Tsybul and Karpukhina (1991) stated that the activity of auxins and cytokinins increased by vernalization and this led to changes in the rate of nutrition and growth.

Moreover, Brown and Menary (1994) found that GA concentration increased during vernalization. In contrast, a reverse pattern was observed in response to vernalization with all Atonik or BA concentrations. Thus, these treatments led to a general progressive increase in total auxins, GA_3 and different cytokinins fractions while ABA significantly decreased (Table 7). In this connection, Malan et al. (1994) found that, BA increase cytokinins availability in *Leucospermum* 'Red sunset'. Feito et al. (1995) concluded that, exogenously applied growth regulators may act directly or through changes in endogenous hormones. They also suggested that, the different uptake and metabolism of BA lead to an increase in cytokinins activity.

Conclusion

In conclusion, the application of Atonik or BA under the influence of vernalization, especially 1000 ppm Atonik and 50 ppm BA play an important role in induction of flowering *S. lycopersicum.* Furthermore the determined metabolic responses (pigment content, carbohydrate content, nitrogenous fractions and ions content) and growth parameters (root length, root fresh weight, root dry weight, shoot length, number of nodes, number of leaves, total leaf area, shoot fresh and dry weights and relative water content) as well as growth regulators

Table 6. Effect of vernalization and different cocentrations of Atonik or benzyl adenine on potassium, sodium and calcium contents, in mg/g dry weight of *Solanum lycopersicum* plant.

Stage	Treatment	Root			Shoot		
		K^+	Na^+	Ca^{++}	K^+	Na^+	Ca^{++}
Initial	Control	15.163	49.706	12.030	24.820	11.366	14.716
	Vernalized	18.956*	56.98*	14.73*	29.03*	13.466*	19.973*
	LSD	0.933	4.606	0.792	1.031	0.867	1.048
Stage I	Control	17.320	60.476	14.880	29.063	13.800	22.570
	Vernalized	20.386*	65.78*	17.24*	31.88*	15.843*	26.076*
	Vernalized+Atonik 250	24.08*	72.52*	20.22*	34.763*	17.426*	27.22*
	Vernalized+Atonik 500	28.313*	75.571*	22.293*	40.336*	19.683*	29.336*
	Vernalized+ Atonik 1000	32.12*	81.586*	27.58*	46.42*	22.79*	34.146*
	Vernalized+BA 25	22.303*	70.466*	18.813*	32.616*	17.133*	25.576*
	Vernalized+BA 50	24.75*	79.686*	21.38*	39.208*	20.513*	28.586*
	Vernalized+BA 100	17.526	66.643*	19.153*	32.606*	16.323*	22.823
	LSD	1.189	3.350	1.130	1.258	0.716	1.400
Stage II	Control	19.123	70.770	18.070	32.860	14.984	25.660
	Vernalized	21.58*	76.55*	21.213*	37.433*	17.41*	27.736*
	Vernalized+Atonik 250	24.52*	79.486*	24.356*	40.776*	20.513*	30.78*
	Vernalized+Atonik 500	27.073*	83.096*	28.266*	45.573*	24.32*	36.406*
	Vernalized+ Atonik 1000	31.303*	89.616*	34.096*	50.066*	27.736*	41.293*
	Vernalized+BA 25	21.933*	81.133*	21.883*	35.926*	21.246*	30.373*
	Vernalized+BA 50	25.856*	88.02*	25.633*	40.95*	24.466*	37.616*
	Vernalized+BA 100	18.463	73.046	20.253*	35.62*	19.05*	29.056*
	LSD	1.098	2.497	1.041	1.669	0.831	1.396

(*) = Significant increase or decrease at 0.05 LSD.

Table 7. Effect of vernalization and different concentrations of Atonik or benzyl adenine on growth regulators content, in µg/g fresh weight of *Solanum lycopersicum* plant in stage I.

Treatment	Total auxins	GA_3	ABA	Zeatin	Kinetin	Benzyladenine
Control	112.333	9.946	34.6	12.306	5	10.103
Vernalized	72.666*	7.13*	48.333*	8.566*	1.959*	6.76*
Vernalized +Atonik 250	132.48*	13.036*	28.666*	14.736*	6.82*	13.263*
Vernalized +Atonik 500	154.133*	18.7*	21.846*	18.57*	9.29*	17.04*
Vernalized + Atonik 1000	191.453*	23.266*	15.6*	22.073*	11.7*	19.416*
Vernalized + BA 25	121.813*	12.886*	29.666*	14.496*	7.846*	22.373*
Vernalized + BA 50	148.503*	19.793*	20.233*	19.183*	9.626*	26.946*
Vernalized + BA 100	155.776*	8.533*	36.223	13.296	6.914*	37.2*
LSD	8.077	0.948	3.318	0.968	0.598	0.898

(*) = Significant increase or decrease at 0.05 LSD.

(auxins, gibberellins, ABA and cytokinins) appeared to be a factor of promoting flowering. This conclusion is supported by Pharis and King (1985) who stated that, the transition from the vegetative to the flowering condition may actually be controlled by changes in the levels of endogenous growth hormones such as auxins,

gibberellins and cytokinins or by the balance between these hormones.

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