*J. Plant Production, Mansoura Univ., Vol. 7 (2):185 - 195, 2016*

# CHILLING TOLERANCE ENHANCEMENT IN TOMATO (*Solanum lycopersicum*) PLANT BY SPRAYING OF SOME ANTI-STRESS COMPOUNDS.

**Hoda I. Ahmed ; Abeer I. Shabana and A. Y. Elsayed**
**Vegetable Res. Dept., Hort. Res. Institute, Agric. Res. Center, Agric. Ministry, Egypt.**

## ABSTRACT

Two field experiments were conducted during two successive winter seasons of 2012/2013 and 2013/2014 at El-Baramoun farm, Mansoura Horticulture Research station to study the effect of spraying by ATP (100ppm), amino acids (2.5ml/l), glycinebetaine (50ppm), salicylic acid (20ppm), Zinc (100ppm) and Boron (50ppm) compared with tap water (control treatment) on vegetative growth parameters, photosynthetic pigments, mineral constituents, yield components and fruit quality of tomato cv. Super strain B grown under low temperature conditions. Significant increases in vegetative growth parameters and photosynthetic pigment contents were recorded relative to corresponding control treatment. ATP treatment was more pronounced in these increments. Marked increases were observed in N, P, K, Ca, Mg and Zn percentages as a result of all applied treatments. However, Zn treatment decreased N and P percentages and gave the highest percentage of Zn during both seasons. B treatment at 50ppm had the highest fruit set percentage, while ATP treatment had the highest yield per plant, early yield and total yield per feddan. In addition, all spraying treatments significantly enhanced tomato fruit quality characters (TSS, TA, VC and lycopene), except for salicylic acid treatment which had no effect on TA (tetratable acidity) relative to control plants. The behavior of most studied traits was not changed from season to other except Chl A, N%, Ca%, F set%, YP, TSS and lycopene indicating the magnitude of the environmental factors on the genetic expression of these traits as controlled by more than one gene. In view of the obtained results, it was found that vegetative growth, yield and fruit quality of tomato plants cv. Super strain B could be enhanced under cold stress condition by spraying by anti-stressor under study (ATP, amino acid, glycinebetain, salicylic acid, Zn or B). It is worthy to mention that ATP spraying treatment at 100 ppm was the most optimum treatment.

**Keywords:** *Lycopersicum esculentum,Mill*, cold stress, ATP, glycinebetain, amino acids, salicylic acid,Zn, B.

## INTRODUCTION

Tomato (*Solanum lycopersicum*) is one of the most important horticultural crops grown in Egypt. It has a great importance as fresh vegetable besides it tops the list of canned vegetables. Tomato as a warm season perennial is injured when exposed to non freezing low temperature (below 12°C) ( Sakveit and Morrris, 1990). Plants of tropical and subtropical origins are sensitive to chilling stress and lack the mechanism of cold acclimation. Generally, plants differ in their tolerance to chilling (0-15°C) and freezing (<0°C) temperatures. Low temperature may affect several aspects of crop growth; viz., survival, cell division, photosynthesis, water transport, growth and finally crop yield. In addition, the cellular changes induced by either high or low temperature include responses those lead to the excess accumulation of toxic compounds, especially reactive oxygen species (ROS). The final result of ROS accumulation is oxidative stress (Suzuki and Mittler,2006). Over accumulation of ROS damage almost all cell components including membrane lipids, chloroplasts, pigments, enzymes and nucleic acids ( Goud and Kachole, 2011). Low temperature affects the plants in every stage of life starting from germination up to maturity. Exposure to temperatures below 13°C may inhibit fruit-set (Atherton and Rudich 1986), while extended exposure to temperatures below 6°C can kill plants. Thus, a serious reduction in tomato fruit yield and quality was observed in winter and early summer seasons every year in Egypt where level of night temperature drops several times below 10°C. Therefore, tomato and other sensitive plants should be exogenously applied by antioxidants and /or stimulants to be capable of tolerating stresses. These applications may offer an economical and simple method for low temperature protection.

Herein, it is beneficial to review the advantageous roles of the assumed treatments i.e., ATP, amino acids, glycinebetaine, salicylic acid, B and Zn. ATP (adenosine triphosphate) is a ubiquitous energy source, but acts extracellularly as a neurotransmitter. ATP and other nucleoside triphosphates not only drive energy- dependent reactions inside cells, but can also function outside the plasma membrane in the extracellular matrix, where they function as agonists that can induce diverse physiological responses without being hydrolyzed. This external role of ATP is well established in animal cells but only recently has become apparent that extracellular ATP (eATP) can also function as a signaling agent in plants (Roux and Steinebrunner, 2007). Recent data have shown that eATP and other nucleotides can induce an increase in the cytosolic Ca concentration and diverse downstream changes that influence plant growth and defense responses (Demidchik et. al., 2003).

Amino acids have been considered as precursors and constituents of proteins and other nitrogen compounds e.g., nucleic acids. Plants subjected to stress show accumulation of proline and other amino acids. The role played by accumulated amino acids in plants varied from acting as osmolyte, regulation of ion transport, modulating stomatal opening and detoxificacation of heavy metals. Amino acids also affected synthesis of some enzymes, gene expression and redox-homeostasis (Rai,2002 ).

Meanwhile glycinebetaine (GB) is synthesized in plants at elevated rates in response to abiotic stresses. Although Tomato plants do not naturally accumulate

(GB) (Wyn Jones and Storey 1981), exogenously applied GB increases their tolerance to stress from salt and drought (Mäkelä *et. al.*, 1998, Mäkelä *et. al*., 1999) and chilling (Park *et. al.,* 2004). Eung-Jun (2006) stated that exogenously applied GB is effectively taken up through the tomato leaf surface and then translocated into different organs. A relatively low endogenous level of GB (>0.09 μmol g–1 FW) in treated plants can provide sufficient protection against chilling temperatures. The majority of the GB is localized to the cytosol, with only a small amount being translocated into the chloroplasts and probably other sub cellular compartments. This action effectively protects the photosynthetic apparatus and enhances chilling tolerance in treated plants. Interestingly, large amounts of GB can be found in meristematic tissues, including the shoot apices and flower buds of treated plants. The high levels in these tissues may be critical for plant survival and enhanced recovery of growth after release from chilling temperatures. Finally, exogenous, foliar application of GB increases levels of $H_2O_2$ catalase gene expression and catalase activity. In addition to a possible direct protective effect on macromolecules such as membranes and proteins, it is likely that the induced chilling tolerance conferred by exogenously applied GB may result from the induction of $H_2O_2$-mediated antioxidant mechanisms, e.g. enhanced catalase expression and catalase activity.

On the other hand, salicylic acid (SA) acts as an endogenic phytohormone from phenolic compounds (among the group of ortho hydroxyl benzoic acid), having the ability of antioxidant defense system and regulates various physiological and biochemical processes in plant such as: stomata conductivity of photosynthesis pigments activities (Hayat *et al.,* 2010), maintenance of tissue water contents and reduced membrane permeability (Farooq *et.al.,* 2008), adjustment the activity of antioxidant enzymes (Carvalho *et al*., 2011) and tolerance to environmental stresses (Kabiri *et al.,* 2012). In addition, Sakhabutdinova *et.al.,* (2003) reported that salicylic acid treatments maintain IAA and cytokinin levels in the plant tissues, which enhanced the cell division and dry weight. Miura and Tada (2014) stated that the effects of SA on the physiological processes of plants depend on its concentration, type of plant, the stage of plant growth and environmental conditions. In general, low concentrations of SA may enhance the antioxidant capacity and tolerance to abiotic stresses but high concentrations of SA may cause cell death or susceptibility to abiotic stresses (Hara *et al.,* 2012). Salicylic acid is an important signaling molecule in plants, improved chilling tolerance and synchronous emergence of maize by activation of antioxidants (Farooq et al., 2008). Furthermore, Miura and Tada (2014) suggested that cold signaling and SA signaling may be interrelated and that the effect of SA on cold tolerance may be species-specific and dependent on the concentration and period of application.

Micronutrients also play an important role in tomato production. Among the micro elements, boron and zinc which play an important role directly and indirectly in improving the yield and quality of tomato in addition to tolerance of various diseases and physiological disorders (Magalhaes et. al., 1980). Zinc plays an important role in chlorophyll formation, cell division, meristematic activity of tissue expansion of cell and formation of cell wall. Zinc application also helps in increasing the uptake of nitrogen and potash. Application of zinc sulphate, copper sulphate and ammonium molybdate stimulates chlorophyll synthesis and fruit quality of tomato (Kalloo, 1985). Kumar, (2003) stated that zinc is an important micronutrient that is closely involved in the metabolism of RNA and ribosomal content in plant cells, also it leads to stimulate carbohydrates, proteins and the DNA formation. It is required for the synthesis of tryptophan, a precursor of IAA, which acts as a growth promoting substance. Zn has also shown to have an important role in photosynthesis and related enzymes which is resulted in increasing sugar and decreasing acidity (Abedy, 2001). Boron plays a major role in plant vital activities such as cell division, leaf and flower bud formation, glucose and hydrocarbons metabolism and transport, root growth, cell wall formation and material transportation between cells ( Majid, et.al.,2012).

Finally, this work aimed to investigate the role of some exogenous applications in enhancement of growth, photosynthetic pigments, mineral composition, yield quantity and quality of tomato plant grown under low temperature stress conditions.

## MATERIALS AND METHODS

Tow field experiments were conducted at El-Baramoun farm, Mansoura Horticulture Research Station during two successive winter seasons of 2012/2013 and 2013/2014 to study the effect of foliar application by anti-stress compounds i.e., ATP, amino acids mixture, glycinbetaine, salicylic acid, boron and zinc compared with tap water. On 1$^{st.}$ week of December, tomato at 40 day after sowing were transplanted at 30 cm apart on one side of ridge 3.5m long and 1m width with experimental unit area of 10.5m$^2$. During this experiment, plants were grown in the first three months under field low temperature conditions where level of night temperature drops several times below 10$^{\circ}$C (Table1a). A Randomized Complete Block Design (RCBD) of three replicates was adopted in both seasons. Plants sprayed four times with different assigned treatments, the first one was at 20 days after transplanting and repeated each 10 days. All cultural practices were performed as recommended. Air temperature during both seasons of this work is presented in Table 1a.

**Table (1a): Monthly air temperature mean in El-Mansoura during seasons of 2012 /2013 and 2013/2014.**

| Months | Air temperature °C | | | |
|---|---|---|---|---|
| | 2012/13 | | 2013/14 | |
| | Maximum | Minimum | Maximum | Minimum |
| November | 24.6 | 12.5 | 25.7 | 13.7 |
| December | 21.6 | 9.5 | 22.2 | 9.7 |
| January | 18.7 | 8.3 | 17.2 | 7.8 |
| February | 19.9 | 9.6 | 20.6 | 8.7 |
| March | 27.5 | 17.7 | 28.1 | 18.9 |
| April | 32.3 | 19.9 | 29.6 | 20.1 |

Treatments were as follows; control treatment was sprayed only with tap water, ATP at 100 ppm, amino acids mixture at 2.5ml/l, glycinebetaine (Sigma chemical Co., U.S.A.)at 50ppm, salicylic acid at 20ppm, boron at 50ppm in form of boric acid(16% B)and zinc at 100ppm in form of zinc citrate(12% Zn). ATP is a chemical agent(Adenosine-tri-phosphate) obtained from El- Gommhoria Co., Egypt in powder crystallized form kept as it and as stock solution at 4°C.

Amino acid composition: A soluble liquid, Spanish compound (delfan) was obtained from Techno green group Cairo, Egypt, was used as a source of amino acids mixture with characteristics as follow: Free amino acids (34.5% w/w); Total nitrogen (4.3% w/w); Organic nitrogen (3% w/w); Organic carbon:(9% w/w); Organic matter (20% w/w).

**Table(1b): Amino acids content (g. amino acids/ 100g muestra).**

| Amino acids | content | Amino acids | content | Amino acids | content |
|---|---|---|---|---|---|
| Aspartic | 2.3 | Glutamic | 4.2 | Serine | 2.8 |
| Glycine | 4.6 | Histidine | 0.3 | Argnine | 2.6 |
| Threionine | 1.2 | Alanine | 2.5 | Proline | 2.8 |
| Tyrosine | 0.9 | Cystine | 0.2 | Valine | 1.8 |
| Methionine | 0.2 | Iso-Leucine | 1.1 | Leucine | 2.1 |
| Phenyl-alanine | 1.1 | Hidroxiproline | 2.7 | Lysine | 1.1 |

**Data Recorded:**

**1-Plant growth and photosynthetic Pigments:**

Three plants from each treatment were randomly taken at 55 days after transplanting and the following data were recorded: plant height, leaf area per plant and dry weight of whole plant. Chlorophyll A and B were determined as described by Wettstein (1957).

**2-Chemical analysis:**

Nitrogen was determined in dry matter of leaves at 55 days after transplanting by using wet digestion according to Piper (1947), using microkjeldahl (Horneck and Miller, 1998). Phosphorus was determined calorimetrically according to Sandell (1950). Potassium was determined according to Horneck and Hanson (1998). Calcium and magnesium were determined according to Jackson (1967). Zinc was determined by using atomic absorption spectrophotometer (Perkin Elmer 3110) as described by A.O.A.C. (1990).

**3- Fruit set percentage and fruit yield:**

At flowering stage, fruit set percentage was calculated according to the following equation:

$$\text{Fruit set \%} = \frac{\text{n. of fruit set in the flower clusters of the plant}}{\text{total n. of flowers anthesis in these clusters}} \times 100$$

At harvesting time yield expressed as number of fruits per plant and yield per plant (kg) were recorded. Total yield and early yield (the sum of the first three pickings) as kg/plot were recorded, then calculated as ton/fed.

**4- Fruit quality:**

At breaker stage, ten ripe tomato fruits/plot were picked and used for determination of TSS% by hand refract meter and vitamin C, titratable acidity and lycopene according to A.O.A.C. (1990).

**5- Individual and combined analysis of variance**

**Statistical analysis:**

The Individual and combined data for two seasons were subjected to analysis of variance and the means were compared using the Least Significant Difference test (L.S.D.) at 5% and 1% levels according to Snedecor and Cachran (1980).

## RESULTS AND DISCUSSION

**1-The mean performance of growth characters and chlorophyll contents**

All applied treatments significantly increased different estimated characteristics i.e., plant height, leaf area and dry weight as well as chlorophyll A and B of tomato compared with those of the control during early seasons of 2012/2013 and 2013/2014 which cold stress occurs. The exceptions were with plant height in case of salicylic acid (20ppm) and leaf area in case of B (50ppm) in the second season which the increase did not reach to the level of significance at 5% (Table 2). However, ATP (100ppm) was more superior in increasing all studied characters during both seasons followed by amino acids (2.5ml/L). Data in the same Table illustrate that photosynthetic pigments i.e., chlorophyll A and B were significantly increased by all treatments and the highest records were obtained with ATP and zinc treatments, respectively in both seasons of this study, except chlorophyll A in the second season since the highest records were given by ATP and salicylic acid, respectively.

**Table (2): Effect of different applied treatments on vegetative growth characters and photosynthetic pigments of tomato grown under low temperature condition.**

| Treat. | | Plant height (cm) | | | Leaf area/plant ($cm^2$) | | | Dry wt./plant (gm) | | | Chlorophyll A (mg/gfw) | | | Chlorophyll B (mg/gfw) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1st s | 2nd s | mean | 1st s | 2nd s | mean | 1st s | 2nd s | mean | 1st s | 2nd s | mean | 1st s | 2nd s | mean |
| Control | | 36.8 | 38.3 | 37.57 | 994.3 | 811.4 | 994.4 | 8.31 | 9.87 | 9.088 | 2.63 | 2.44 | 2.535 | 1.47 | 1.25 | 1.360 |
| ATP | | 47.3 | 49.2 | 48.28 | 2722.0 | 2613.8 | 2722.0 | 37.93 | 35.60 | 36.77 | 4.69 | 4.52 | 4.615 | 2.70 | 2.73 | 2.715 |
| A. acids | | 45.5 | 45.4 | 45.45 | 2510.0 | 2693.5 | 2513.5 | 31.27 | 34.70 | 32.99 | 3.65 | 3.61 | 3.630 | 2.34 | 2.21 | 2.227 |
| G b. | | 42.8 | 41.7 | 42.25 | 2275.0 | 2204.8 | 2275.0 | 25.27 | 27.47 | 26.37 | 3.66 | 3.49 | 3.573 | 2.03 | 2.01 | 2.022 |
| Sa. acid | | 41.8 | 40.9 | 41.35 | 2176.0 | 2204.9 | 2176.0 | 27.56 | 29.20 | 28.38 | 3.74 | 3.60 | 3.667 | 2.13 | 2.13 | 2.132 |
| Zn | | 44.0 | 42.7 | 43.28 | 2290.0 | 2804.8 | 2290.0 | 30.78 | 31.17 | 30.97 | 3.84 | 3.48 | 3.658 | 2.49 | 2.49 | 2.488 |
| B | | 45.1 | 43.5 | 44.30 | 1433.3 | 817.0 | 1433.2 | 22.32 | 22.50 | 22.41 | 3.14 | 3.03 | 3.088 | 2.35 | 2.31 | 2.333 |
| LSD | 5% | 3.696 | 2.011 | 2.749 | 80.98 | 122.1 | 100.5 | 1.139 | 3.246 | 2.573 | 0.126 | 0.188 | 0.153 | 0.108 | 0.151 | 0.139 |
| | 1% | 5.560 | 3.026 | 3.994 | 121.8 | 183.7 | 146.1 | 1.713 | 4.883 | 3.739 | 0.190 | 0.283 | 0.222 | 0.162 | 0.226 | 0.202 |

**control: tap water ; ATP(100 ppm); A. acids: amino acids mixture (2.5ml/l); G.b.: glycinebetaine (50 ppm), Sa. Acid: salicylic acid ( 20ppm), Zn: zinc (100 ppm); B: boron (50ppm ).**

Meanwhile, progressive depression in all morphological characters under study was noticed in untreated control plant and this is in agreement with Farooq *et.al*., (2008) who suggested that the adverse effects of stress on plant height and root length may be due to its effects on meristimatic cell division and elongation as well as root penetration. In addition, Rezaei *et.al*., (2012) reported that at any time of crop development, stress reduced crop photosynthesis rate, by which the total assimilates are being available to the crop. Thereby, under stress, cell expansion is inhibited, which expresses itself as reduction of leaf area expansion. Moreover, Fathy *et.al*., (2000) stated that during cold stress severe disturbances in respiration pathways and reduction in ATP content occur during the first few days of growth at low temperature as a result of P and sugars shortage. So, an actually agent ATP demanded is required by plants of limited chilling tolerance capacity as tomatoes.

For amino acids, alternative routes of IAA synthesis exist in plants, all starting from tryptophan (Phillips,1971). Hass (1975) reported that the biosyntheses of cinamic acids (which are the starting materials for the synthesis of phenols) are derived from phenylalanine and tyrosine. Tyrosine is hydroxy phenyl amino acid that is used to build neurotransmitters and hormones. Organic nitrogenous compounds are the building blocks in the synthesis of proteins, which are formed by a process in which ribosomes catalyze the polymerization of amino acids. Amino acids are particularly important for stimulation cell growth. They can serve as a source of carbon and energy as well as protect the plants against stress. Amino acids also function in the synthesis of other organic compounds, such as protein, amines, purines and pyrimidines, alkaloids, vitamins, enzymes and terpeniods ( Attoa *et. al.,* (2002).

Salicylic acid is a key signaling molecule in induction of plant defense mechanism and reduces symptoms of environmental stress as well as regulates plant growth and development (Horváth *et al*., 2007). The increases in fresh and dry matter of stressed plants in response to SA might be related to the induction of antioxidant response that increased the tolerance of plants to damage (Gunes *et al.*, 2005). SA treatments increased the level of cell division by stimulating the mitotic system of the apical meristem of seedling roots which caused an increase in plant growth (Sakhabutdinova *et al*., 2003). Moreover, Salwa *et. al.,* (2015) found that SA treatment increased the chlorophyll,carotenoid contents, improved the photosynthetic efficiency and enhanced photosynthetic rate, dry weights and maintained membrane integrity, leading to improvement of plant growth in tomato plants under cold stress conditions.

On the other hand, accordingly to beneficial effects of Zn known to enhance cell division and differentiation, viability and repeatability of the reproductive organs and induce an active and balanced hormonal status of higher IAA and GA's content vs. low ABA and ethylene within such reproductive and other plant organs (Domingo et al., 1990). Also, it plays a defensive protective role against adverse effects of higher temperature via its antioxidant and gene regulatory functions. Moreover, it was reported that zinc enhances translocation of bioassimilates and nutrients within plant tissues as they activate the membrane transporter enzymes. In addition, foliar application of zinc improved growth and productivity of sweet pepper crop (Cakmak and Marschner, 1988). Singh and Tiwari (2013) obtained that zinc application increased chlorophyll content. Mohsen (2013) demonstrated that by adding Zn and Fe, leaf surface, vegetative growth, net photosynthetic rate and chlorophyll content of plants increased. Zinc is a component of carbonic anhydrase, as well as several dehydrogenases and auxin production which in turn will enhance plant growth.

Also, boron is important in energy storage or structural integrity functions including sugar transport, cell wall synthesis, signification and cell wall structure, carbohydrate, IAA and phenol metabolism and respiration **(**Bondok, 1996). This proved the efficiency of the anti-oxidants in protection of the plants against low temperature inducible toxic oxygen free radicals. Similarly, Sajid *et. al.*, (2013) and Singh and Tiwari (2013) also reported somewhat similar findings regarding tomato plant height in response to different micronutrients (Zn and B) application as a foliar feeding.

**2- Chemical analysis:**

Concerning the effect of various treatments on mineral constituents i.e., N, P, K, Ca, Mg and Zn, Table 3 clearly shows that all treatments increased mineral contents of tomato leaves under cold stress condition relative to those of control plants during the two seasons of this work, whereas the only exception was with N and P percentages since they were decreased as a result of Zn treatments in both seasons of this work. It is also obvious that ATP was more superior in increasing all mineral concentrations under study, except for zinc content which had the highest records in case of Zn application during both seasons.

Fathy *et.al.,* (2000) demonstrated that under cold stress condition, untreated plants might be in energy starved case due to their poor content of P and sugars those known to be involved in energy metabolism. In addition, the exposure of limited chilling capacity plants such as tomato to low temperature stress lead to depletion in carbohydrate reserves, sever decrease in P uptake and more expenditure of ATP (Sobczyk *et. al.,* 1985) and consequently a restriction in mineral uptake and carbohydrate synthesis occurs(ATP dependent processes).

The beneficial effect of exogenous ATP treatment on mineral, carbohydrates and sugar content might be explained as ATP activated $H^+$-ATP-ase membrane pumps of ions absorption, translocation and retention (Palta, 1990). Also, ATP via its cytokinins function–might be increased water and nutrients uptake and photo-metabolites translocation by controlling sink-source relationship (Fathy *et. al.,* 2000).

Additionally, the main function of anti-oxidants such as SA was protective of cell membranes and their binding transporter proteins ($H^+$-ATP–ase membrane pumps), maintained their structure and function against the toxic and destructive effects reactive oxygen species (ROS) during stress, in turn, more absorption and translocation of minerals (Dicknson *et .al.,*1991). Moreover, Dube *et. al,*. (2003) obtained that the highest zinc content of leaves with the highest rate of zinc, however, micronutrient fertilizing decreased P and N content in leaves. Zinc decreases phosphorous content because there is competitive effect between phosphorous and zinc in uptake of ions. The increase obtained with Zn treatment might be attributed to its role in preventing the formation of free radicals ($O_2$, $H_2O_2$, OH ….*etc.*) and thereby, the membrane leakage chlorosis and necroses of leaves. Besides, it may prevent the oxidative degeneration of IAA and consequently increases the level of IAA in plants. Such increase causes an enhancement of plant growth and mineral nutrients uptake and translocation or partially due to that sugar acts as an osmoregulator in plant cell; the process that participates in enhancing mineral uptake and translocation in plants and consequently the higher concentration of minerals in plant tissues(Marschner ,1995) .

**3- Fruit set percentage and fruit yield**

It is quite clear from Table 4 that fruit set percentage and number and weight of fruit per plant as well as early and total yield per fedden were significantly increased as a result of all applied treatments of the two seasons. On the contrary, the control plants (sprayed by tap water) were strongly stressed. It is also observed that treatments considerably differed among of them in their effects since great enhanced fruit weight per plant and early and total yield per feddan were obtained by ATP treatment followed by B treatment, while the highest fruit set percentage and number of fruits per plant were obtained by B treatment.

**Table(3):Chemical composition of tomato leaves as affected by different applied treatments under low temperature condition**.

| **Treat.** | **N %** | | | **P %** | | | **K%** | | | **Ca%** | | | **Mg%** | | | **Zn (ppm)** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1st s** | **2nd s** | **mean** | **1st s** | **2nd s** | **mean** | **1st s** | **2nd s** | **mean** | **1st s** | **2nd s** | **mean** | **1st s** | **2nd s** | **mean** | **1st s** | **2nd s** | **mean** |
| Control | 2.51 | 2.70 | 2.758 | 0.160 | 0.150 | 0.155 | 1.70 | 1.71 | 1.707 | 1.35 | 1.32 | 1.337 | 0.400 | 0.398 | 0.399 | 75.85 | 73.27 | 74.56 |
| ATP | 3.34 | 2.87 | 3.105 | 0.513 | 0.500 | 0.507 | 3.08 | 3.06 | 3.072 | 3.11 | 3.07 | 3.092 | 0.736 | 0.712 | 0.724 | 166.53 | 166.53 | 166.5 |
| A. acids | 3.25 | 2.90 | 3.075 | 0.233 | 0.321 | 0.277 | 1.80 | 1.90 | 1.850 | 2.00 | 1.95 | 1.975 | 0.461 | 0.501 | 0.481 | 118.21 | 108.60 | 113.4 |
| G b. | 3.19 | 2.80 | 2.993 | 0.347 | 0.293 | 0.320 | 1.96 | 1.91 | 1.935 | 2.20 | 2.11 | 2.155 | 0.440 | 0.450 | 0.445 | 115.06 | 109.33 | 112.2 |
| Sa. acid | 3.10 | 2.65 | 2.878 | 0.400 | 0.420 | 0.410 | 1.80 | 1.82 | 1.812 | 2.13 | 2.01 | 2.070 | 0.631 | 0.599 | 0.615 | 112.75 | 110.30 | 111.5 |
| Zn | 2.51 | 2.49 | 2.498 | 0.100 | 0.109 | 0.104 | 2.01 | 1.97 | 1.992 | 2.91 | 2.55 | 2.732 | 0.451 | 0.473 | 0.462 | 176.53 | 181.50 | 178.9 |
| B | 3.00 | 2.67 | 2.833 | 0.430 | 0.400 | 0.415 | 2.05 | 2.01 | 2.030 | 2.70 | 2.40 | 2.552 | 0.616 | 0.609 | 0.612 | 160.29 | 157.23 | 159.0 |
| LSD 5% | 0.174 | 0.076 | 0.125 | 0.041 | 0.015 | 0.044 | 0.113 | 0.110 | 0.108 | 0.164 | 0.070 | 0.117 | 0.015 | 0.015 | 0.000 | 7.575 | 4.092 | 6.986 |
| LSD 1% | 0.262 | 0.114 | 0.181 | 0.062 | 0.022 | 0.064 | 0.170 | 0.165 | 0.157 | 0.247 | 0.105 | 0.169 | 0.022 | 0.022 | 0.000 | 11.396 | 6.156 | 10.151 |

**control: tap water; ATP(100ppm); A.acids: amino acids(2.5ml/l); Gb.: glycinebetain (50 ppm); Sa. Acid:salicylic acid( 20ppm); Zn: zinc (100 ppm); B: boron(50ppm).**

**Table (4): Tomato fruit yield as affected by different applied treatments under low temperature condition**.

| Treat. | | Fruit set% | | | No.F/plant | | | Yield/plant (kg) | | | Early yield ton/fed | | | Total yield ton/fed | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1st s | 2nd s | mean | 1st s | 2nd s | mean | 1st s | 2nd s | mean | 1st s | 2nd s | mean | 1st s | 2nd s | mean |
| Control | | 19.60 | 20.37 | 19.98 | 11.00 | 10.67 | 10.83 | 0.65 | 0.65 | 0.650 | 1.8 | 1.7 | 1.75 | 8.2 | 7.8 | 8.00 |
| ATP | | 50.33 | 56.07 | 53.20 | 21.33 | 22.33 | 21.83 | 1.51 | 1.60 | 1.555 | 4.8 | 4.5 | 4.65 | 20.1 | 20.4 | 22.50 |
| A. acids | | 44.29 | 46.27 | 45.28 | 22.00 | 22.00 | 22.00 | 1.35 | 1.42 | 1.385 | 3.9 | 3.7 | 3.80 | 17.1 | 18.0 | 17.55 |
| G b. | | 38.10 | 40.33 | 39.22 | 22.33 | 20.00 | 21.17 | 1.17 | 1.44 | 1.305 | 3.2 | 4.0 | 3.60 | 15.3 | 18.2 | 16.75 |
| Sa. Acid | | 48.10 | 50.07 | 49.08 | 18.00 | 19.33 | 18.67 | 1.25 | 1.32 | 1.285 | 3.4 | 3.8 | 3.60 | 15.8 | 16.7 | 16.25 |
| Zn | | 47.29 | 51.17 | 49.23 | 20.33 | 20.00 | 20.17 | 1.40 | 1.32 | 1.360 | 3.9 | 3.6 | 3.75 | 17.8 | 16.5 | 17.15 |
| B | | 54.17 | 60.53 | 57.35 | 24.67 | 22.00 | 23.33 | 1.40 | 1.42 | 1.410 | 4.1 | 4.4 | 4.25 | 18.0 | 18.2 | 18.1 |
| LSD | 5% | 2.98 | 2.08 | 2.37 | 0.98 | 1.91 | 1.40 | 0.04 | 0.11 | 0.076 | 0.23 | 0.22 | 0.21 | 0.21 | 0.24 | 0.22 |
| | 1% | 4.48 | 3.12 | 3.45 | 1.47 | 2.88 | 2.04 | 0.06 | 0.16 | 0.111 | 0.35 | 0.33 | 0.31 | 0.32 | 0.35 | 0.31 |

**control:tap water; ATP(100ppm); A.acids:amino acids(2.5ml/l); Gb.: glycinebetain (50 ppm); Sa. Acid:salicylic acid( 20ppm);Zn:zinc(100 ppm);B boron(50ppm).**

The effect of cold stress on flowering and fruit set on the different plant axes had influenced on yield and yield components. Similar results were obtained in tomato (Mady, 2009) with SA and ( Fathy,*et.al*.,2000 ) with ATP under cold stress condition. Moreover, the explanation of yield increase of stressed-plants after application of GB has been proposed to be partly located on the increased net photosynthesis, decreased rate of photorespiration, stomatal conductance, induced more efficient gas exchange (Makela *et al*., 1998) and thus, better availability of carbon for photosynthetic processes and ability to avoid possibly photo-inhibition (Makela *et al*., 1999), water use efficiency (Bergmann and Eckert,1984) and increased chlorophyll content in plants (Whapman *et al.*, 1993). In addition, GB protects, stabilizes and activates the proteins of photosynthetic reactions (Papageourgiou and Murata, 1995). Exogenous application of GB also results in higher yields in the greenhouse and field, mainly due to improved net photosynthesis and growth rates (Mäkelä *et al.* 1998).The soybean crop stability and yield was often increased due to foliar applications of GB when sprayed during the stress( Rezaei*, et. al.*, 2012).

The high fruit set percentage and number of fruits per plant might be owing to the optimum application of boron along with zinc as a foliar feeding (Sajid, *et. al.,* 2013). Nonnecke (1989) also reported similar information regarding fruit set of tomato and enhanced nutrients uptake due to foliar application that resulted in increased assimilation rate percentage. Positive effect of $ZnSO_4$ on fruit number is well documented by Tamilselvi et. al,. (2002), who stated that $ZnSO_4$ is applied to raise fruit number, size and quality and the increase in total fruit yield was due to enhancement of tomato vegetative growth and biosynthesis accumulation consequential by optimal availability of some required nutrients. In addition, Boron is important in pollen germination and pollen tube growth, which is likely to increase fruit set. Therefore, boron fertilization may increase yield. Day (2000) also reported that optimum amount of boron may have promoted flower clusters development. The increase in number and weight of fruit per plant as a result of boron may be attributed to greater photosynthetic activity, resulting the increased production and accumulation of carbohydrates and favorable effect on vegetative growth and retention of flowers and fruits, which might have increased number and weight of fruits. The results of this finding is in agreement with studies done in brinjal crop by Suganiya and Kumuthini (2015)

**4- Fruit quality**

Respecting with fruit quality, data in the Table 5 reveal that most applied treatments increased all quality parameters( TSS, VC, TA and lycopene) and ATP treatment was almost superior in increasing vitamin C, TSS%, acidity % lycopene content in both seasons of this work .The exception was SA in both seasons which had no significant increase on acidity% in both seasons. Moreover, lycopene content also did not reach to the significant level at 5% with glycinebetaine treatment in both early summer seasons of this work.

Huseyin *et .la*.,(2009) stated that foliar applications of SA positively affected TSS and ascorbic acid (AA) content of strawberry fruit, but had no effect on TA of strawberry fruits, while Mady, (2009) stated that SA increased TSS,TA,VC in tomato fruits. Under cold stress condition, Fathy et. al., (2000), Salwa, et. al., (2015) show that TSS levels were increased as a result of ATP and salicylic acid treatment, respectively in tomato fruits. Regarding with Zn and B, Salam et. al.,(2010) reported that acidity content of the fruits which was better for canning performance increased with the increasing boron and zinc levels, while lycopene content was improved by Zn application (Dube et. al., 2003). Resembling results were obtained by Mohsen (2013) in tomato.Zinc has also shown to have an important role in photosynthesis and related enzymes, which will lead to increasing sugar and decreasing acidity (Abedy, 2001).

**Table (5): Tomato fruit quality as affected by different applied treatments under cold condition.**

| Treat. | | V.C (mg/100gfw) | | | TSS (%) | | | Acidity (%) | | | Lycopene (mg/100g fw) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1st s | 2nd s | mean | 1st s | 2nd s | mean | 1st s | 2nd s | mean | 1st s | 2nd s | mean |
| Control | | 17.41 | 16.33 | 16.87 | 3.76 | 4.31 | 4.033 | 0.357 | 0.356 | 0.357 | 4.05 | 4.05 | 4.052 |
| ATP | | 21.56 | 20.77 | 21.16 | 4.50 | 4.55 | 4.527 | 0.387 | 0.446 | 0.446 | 4.65 | 4.99 | 4.817 |
| A. acids | | 20.55 | 19.27 | 19.90 | 4.60 | 4.54 | 4.570 | 0.420 | 0.436 | 0.436 | 4.29 | 4.52 | 4.403 |
| G b. | | 20.10 | 19.50 | 19.80 | 4.46 | 4.55 | 4.505 | 0.400 | 0.400 | 0.400 | 4.10 | 4.06 | 4.078 |
| Sa. acid | | 19.33 | 21.37 | 20.34 | 4.60 | 4.42 | 4.510 | 0.372 | 0.364 | 0.364 | 4.37 | 4.42 | 4.392 |
| Zn | | 20.25 | 20.04 | 20.14 | 4.41 | 4.40 | 4.407 | 0.410 | 0.400 | 0.402 | 4.40 | 4.53 | 4.468 |
| B | | 21.40 | 21.43 | 21.41 | 4. 39 | 4.37 | 4.383 | 0.400 | 0.403 | 0.403 | 4.28 | 4.30 | 4.290 |
| LSD | 5% | 0.138 | 2.405 | 2.308 | 0.025 | 0.065 | 0.044 | 0.015 | 0.015 | 0.014 | 0.052 | 0.135 | 0.098 |
| | 1% | 0.208 | 3.618 | 3.354 | 0.038 | 0.098 | 0.064 | 0.022 | 0.022 | 0.020 | 0.079 | 0.203 | 0.143 |

**control: tap water; ATP(100 ppm); A. acids: amino acids mixture (2.5ml/l); G.b.: glycinebetaine (50 ppm); Sa: salicylic acid ( 20ppm); Zn: zinc (100 ppm); B: boron (50ppm ).**

In conclusion, spraying any of the assumed treatments (ATP, amino acids, glycine-betaine, salicylic acid, Zn or B) on tomato plants cv. Super strain B grown in open field in early summer and winter seasons ,where chilling stress occurs, could improve the yield and quality of fruits as a reflection of enhancing vegetative growth, photosynthetic pigments and mineral constituents. ATP at 100ppm was more effective in ameliorating the adverse effects of chilling.

**5- Individual and combined analysis of variance:**

The analysis of variance and mean squares of vegetative traits, chemical composition, yield and fruit quality were obtained and the results are presented in Table 6. It appeared from the Table that the mean squares of the treatments were highly significant for the majority of studied traits in both seasons except for VC which was only significant in the 2nd season. These results indicated that there were significant differences not only among the exogenous applications, but also among the control and other treatments. The presence of significant differences would indicate that the comparison among the means of treatments would be a valid test.

**Table(6):The individual analysis of variance and mean squares of vegetative traits,chemical composition, yield and fruit quality of tomato affected by different applied treatments under low temperature condition.**

| Traits | Mean Square | | | | | |
|---|---|---|---|---|---|---|
| | Replication. (d.f: 2) | | Treatments (d.f: 6) | | Error (d.f: 12) | |
| | 1st s | 2nd s | 1st s | 2nd s | 1st s | 2nd s |
| PH | 11.25 | 8.583 | 34.50** | 36.67** | 6.451 | 1.911 |
| LA | 11912.3 | 61.68 | 1140814** | 1144135** | 3097.9 | 7045.4 |
| DW | 32.66 | 10.21 | 261.1** | 234.6** | 0.613 | 4.976 |
| Chl A | 0.008 | 0.010 | 1.212** | 1.212** | 0.008 | 0.017 |
| Chl B | 0.009 | 0.032 | 0.471** | 0.654** | 0.006 | 0.011 |
| N % | 0.007 | 0.032 | 0.247** | 0.061** | 0.014 | 0.003 |
| P % | 0.003 | 0.000 | 0.069** | 0.062** | 0.001 | 0.000 |
| K % | 0.003 | 0.011 | 0.657** | 0.622** | 0.006 | 0.006 |
| Ca % | 0.000 | 0.007 | 1.106** | 0.900** | 0.013 | 0.002 |
| Mg % | 0.000 | 0.0001 | 0.048** | 0.0360** | 0.000 | 0.0001 |
| Zn | 1.542 | 198.2 | 3987.9** | 4609.5** | 27.10 | 7.908 |
| F Set % | 1.003 | 3.366 | 398.0** | 522.0** | 4.182 | 2.036 |
| NFP | 0.619 | 0.619 | 59.05** | 49.54** | 0.452 | 1.730 |
| YP(kg/p) | 0.005 | 0.0002 | 0.244** | 0.281** | 0.001 | 0.006 |
| EY(ton/fed) | 0.056 | 0.023 | 2.654** | 2.617** | 0.026 | 0.023 |
| TY(ton/fed) | 0.009 | 0.053 | 43.39** | 49.48** | 0.021 | 0.026 |
| V.C | 0.009 | 0.020 | 5.946** | 9.253* | 0.009 | 2.732 |
| TSS | 0.001 | 0.0041 | 0.252** | 0.0290** | 0.000 | 0.0020 |
| Acid. | 0.000 | 0.0000 | 0.003** | 0.0033** | 0.000 | 0.0000 |
| Lyco. | 0.001 | 0.0053 | 0.120** | 0.3101** | 0.001 | 0.0086 |

**PH:plant height(cm) ; LA :leaf area($cm^2$/plant); DW: dry weight(gm/plant); chl A and B: chlorophyll A and B (mg/g fresh weight); FSet%: fruit set%; NFP: No of fruits/plant; YP: yield/plant(kg); EY: early yield (ton/fed);TY: total yield(ton/fed).**
**** and * significant at 1 and 5% probability levels, respectively by F test.**

The combined analysis over the two seasons as shown in Table (7) revealed that the mean square of treatments for VC. was significant at 5% of probability and not significant for TSS. While the behavior of most studied traits was not changed from season to other except Chl A, N%, Ca%, F set %, YP, TSS and lycopene. Indicating the magnitude of the environmental factors on the genetic expression of these traits as polygenic type controlled by more than one gene like most abiotic stress tolerance (Wahid *et al.*, 2007).

**Table(7):The combined analysis of variance and mean squares of vegetative traits, chemical composition, yield and fruit quality of tomato affected by different applied treatments under low temperature condition over two years.**

| Traits | Replications df = 2 | Treatments df =6 | Years df =1 | Treat.xYears df =6 | Error df =26 |
|---|---|---|---|---|---|
| PH | 19.39 | 68.18** | 0.619$^{ns}$ | 2.996$^{ns}$ | 3.894 |
| LA | 5130 | 2284938** | 9.734$^{ns}$ | 10.66$^{ns}$ | 5208 |
| DW | 32.05 | 490.6** | 10.68$^{ns}$ | 5.055$^{ns}$ | 3.412 |
| Chl A | 0.005 | 2.409** | 0.292** | 0.015$^{ns}$ | 0.012 |
| Chl B | 0.004 | 1.113** | 0.034$^{ns}$ | 0.012$^{ns}$ | 0.01 |
| N % | 0.035 | 0.264* | 0.984** | 0.044** | 0.008 |
| P % | 0.001 | 0.127** | 0.000$^{ns}$ | 0.003** | 0.001 |
| K% | 0.001 | 1.275** | 0.000$^{ns}$ | 0.004$^{ns}$ | 0.006 |
| Ca% | 0.004 | 1.979** | 0.216** | 0.027** | 0.007 |
| Mg% | 0.000 | 0.083** | 0.000$^{ns}$ | 0.001** | 0.000 |
| Zn | 82.95 | 8566** | 74.05$^{ns}$ | 31.87$^{ns}$ | 25.15 |
| F Set % | 3.969 | 913.3** | 112.6** | 6.682$^{ns}$ | 2.901 |
| NFP | 1.143 | 105.1** | 2.381$^{ns}$ | 3.492* | 1.015 |
| YP(kg/p) | 0.003 | 0.507** | 0.041** | 0.018** | 0.003 |
| EY(ton/fed) | 0.074 | 5.004** | 0.077$^{ns}$ | 0.267** | 0.023 |
| TY(ton/fed) | 0.027 | 90.37** | 2.429$^{ns}$ | 2.507** | 0.024 |
| V.C | 0.004 | 13.33* | 0.760$^{ns}$ | 1.869$^{ns}$ | 2.745 |
| TSS | 0.001 | 0.200$^{ns}$ | 0.036** | 0.080** | 0.001 |
| Acid. | 0.0001 | 0.0066** | 0.0000$^{ns}$ | 0.0000$^{ns}$ | 0.0001 |
| Lyco. | 0.002 | 0.402** | 0.117** | 0.028** | 0.005 |

**PH: plant height(cm) ; LA : leaf area($cm^2$/plant); DW: dry weight(gm/plant); chl A and B: chlorophyll A and B(mg/g fresh weight); FSet%: fruit set%; NFP: No of fruits/plant; YP: yield/plant(kg); EY: early yield (ton/fed); TY: total yield(ton/fed).**
**** and * significant at 1 and 5% probability levels, respectively by F test.**

Regarding treatment x years interactions, the analysis of variance shows that the absence of any significant interaction for vegetative traits, pigment contents and fruit set % indicating the stability of our exogenous applications effects on these traits in addition to certain chemical composition as K% and Zn (Table 7). In contrast, this interaction was significant and highly significant for NFP and YP, respectively.

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**تحسين تحمل نباتات الطماطم للبروده بالرش ببعض المركبات المضادة للاجهاد**

**هدى ابراهيم أحمد, عبير ابراهيم عبد الغفار شبانه وأحمد يوسف عبد النبى**
**قسم بحوث الخضر- معهد بحوث البساتين – مركز البحوث الزراعيه**

أجريت تجربتان حقليتان على نبات الطماطم صنف سوبر سترين بى خلال العروه الشتويه لموسمى ٢٠١٣/٢٠١٢ و ٢٠١٤/٢٠١٣ في المزرعه البحثيه بالبرامون التابعه لمحطة بحوث البساتين بالمنصوره وذلك لمقارنة تأثير الرش بمحلول أدينوزين تراى فوسفات (١٠٠ جزء فى المليون), والأحماض الأمينيه (٢.٥ مل/لتر)، الجلايسن بيتايين (٥٠ جزء في المليون)، حمض السلسليك (٢٠ جزء فى المليون)، عنصر الزنك (١٠٠ جزء فى المليون) وعنصر البورون (٥٠ جزء فى المليون) مقارنة بالرش بالماء على كل من صفات النمو الخضرى، محتوى الأوراق من الكلوروفيل والعناصر المعدنيه وعلى نسبة العقد فضلا عن كمية وجودة محصول الثمار الناتجه فى ظروف انخفاض درجة حرارة الجو. وقد أظهرت النتائج زياده معنويه في جميع صفات النمو الخضرى ومحتوى الأوراق من كلوروفيل أ و ب مقارنة بمعاملة المقارنه وكان الرش بالأدينوزين تراى فوسفات الأكثر تفوقا بينما تباينت باقى المعاملات فيما بينها لتعكس درجات متفاوته من تحمل النباتات للبروده. كما أظهرت النتائج زياده ملحوظه فى المحتوى المعدنى للنبات من النيتروجين، البوتاسيوم، الفوسفور، الكالسيوم، الماغنيسيوم والزنك بينما أدت معامله الرش بالزنك (١٠٠ جزء فى المليون) الى الحصول على أعلى محتوي من الزنك وأقل محتوي من الفوسفور والنيتروجين خلال موسمى الزراعه. أعطت معامله الرش بالبورون بتركيز ٥٠ جزء فى المليون أعلى نسبة عقد بينما كان الرش بالأدينوزين تراى فوسفات الأكثر تفوقا في وزن الثمار للنبات والمحصول المبكر و الكلى للفدان. كما حسنت جميع المعاملات المدروسه من صفات جودة ثمار الطماطم(المواد الصلبه الذائبه الكليه-حمض الأسكوربيك- الحموضه- الليكوبين) الناميه تحت تأثيراجهاد الحراره المنخفضه بالمقارنه بالنباتات الغير معامله(الكنترول) فيماعدا حمض السلسليك الذى لم يظهر أى تأثير على الحموضه بالثمار مقارنة بالكنترول. لم يتأثر سلوك معظم الصفات المدروسة من موسم لاخر فيما عدا محتوى الأوراق من كلورفيل A, المحتوى النتروجينى, نسبة الكالسيوم,نسبة العقد, محصول النبات ,نسبة المواد الصلبة الذائبة و الليكوبين وربما يرجع الى أهمية العوامل الخارجية في التعبير الوراثى لهذه الصفات ذات الطبيعة الكمية.

وفى ضوء النتائج المتحصل عليها وجد أن رش نباتات الطماطم باحد مضادات الاجهاد مثل أدينوزين تراى فوسفات، الأحماض الأمينيه، جلايسين بيتايين،حمض السلسليك، الزنك أو البورون أدى الى تحسين النمو الخضرى مع زيادة المحصول كما و نوعا تحت ظروف الحراره المنخفضه.و جدير بالذكر أن أكثر المعاملات تفوقا كانت معاملة أدينوزين تراى فوسفات بتركيز ١٠٠ جزء فى المليون.