Minufiya J. Agric. Res. Vol.35 No. 2:663-685 (2010) "http://agri.menofia.edu.eg/megla.html"

PROTECTION OF TOMATO PLANTS AGAINST COLD STRESS BY USING ANTIOXIDANTS, CHILLING HARDENING AND JASMONIC ACID

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(Received: Feb. 3, 2010)

ABSTRACT : ***Two field experiments were carried out under cold weather conditions of winter seasons of 2008 and 2009, to study the effect of some treatments i.e., chilling, Jasmonic acid, salicylic acid and selenium on growth, relative water content, membrane leakage, biochemical constituents, early yield and its components of tomato plants.***

All treatments under study significantly increased fresh and dry weight of leaves, stems and roots, leaf area and net assimilation rate. Moreover, these treatments significantly increased RWC, chlorophyll a , b and (a + b) and compitable osmolytes (proline, total sugars, total amino acids and K^+). Meanwhile, all treatments significantly reduced membrane leakage compared to control plants. Also, the previous treatments significantly increased N and P concentration, early yield, total yield and fruit quality (Vit.C and T.S.S.). The most effective treatments were SA and Se followed by chilling and JA compared with control plants.

The data suggest that SA and Se play an important antioxidant role in protecting tomato plants from cold stress condition, and plants showed a better performance which reflected higher early yield and good fruit quality.

Key Words*: Cold stress, Antioxidants, Selenium, Salicylic acid, Membrane leakage, Catalase, Peroxidase, Phenoloxidase.*

INTRODUCTION

The severe reduction in tomato productions occurs in spring every year in Egypt. It seems that this drop in productivity is a resultant of low night temperature prevailing during January and February which may induce poor fruit-set. Besides frost waves occure almost annually causing in some cases, severe damage for vegetative growth of tomato.

Acclimation to cold stress has been reported to involve the synthesis of proteins, membrane lipids, and metabolites that confirm chilling tolerance (Graham and Patterson, 1982). The mechanism of acclimation has been postulated to involve maintenance of membrane fluidity by increasing lipid unsaturation, and enzymatic protective systems (Levitt, 1980). More recently, a linkage between cold acclimation and resistance to oxidative stress has been postulated (Prasad *et al.,* 1994). Thus, oxidative stress plays a dual role in low-temperature responses, as a source of injury and as a signal to

increase antioxidant defenses. Enhancing cold tolerance was also associated with treatments that increased the level of antioxidants.

Evidence has been reported suggesting that cold stress takes the form of oxidative stress, caused by the stimulation of free oxygen radical production (Kanogwan *et al*., 1997). In chilling sensitive plants such as *Zea mays* L., exposure to low temperatures in the light leade to the peroxidation of membrane lipids and the depletion of antioxidant compounds such as tocopherol, both indications of increased levels of reactive oxygen species (Prasad *et al*., 1994). Under severe stress conditions, however, the antioxidant capacity may not be sufficient to minimize the harmful effect of oxidative injury.

Survival under stressful conditions depends on the plant's ability to perceive the stimulus, generate and transmit signals, and induce biochemical changes that adjust the metabolism accordingly. Therefore, the search for signal molecules that mediate the stress tolerance is an important step towards a better understanding of how plants adjust to an adverse environment.

Attempts towards enhancing cold tolerance in plants has been reported by several workers. Appling Salicylic acid solution to banan seedling (Kong *et al*., 2003) and cucumber plants (Yildrim *et al*., 2008) induced chilling tolerance in both crops. Salicylic acid is known to improve the cold tolerance of plants. Salicylic acid (SA) a natural plant product, combines growth enhancement and antisenescence properties (Raskin, 1995), and is involved in eliciting specific responses to biotic and abiotic stresses. It has been shown that SA provides protection against low-temperature stress in maize (Janda *et al*., 1999 and Horvath *et al*., 2002) and winter wheat plants (Tasgin *et al*., 2003), induces thermotolerance in mustard seedlings (Dat *et al*., 1998), and modifies plant responses to salt osmotic stresses (Borsani *et al*., 2001), drought (Senaratna *et al*., 2000). SA has been shown to accumulate in plants in response to various oxidizing stresses, such as H_2O_2 (Leon *et al*., 1995). Besides the above visual symptoms, it was confirmed by chlorophyll florescence parameters and electrolyte leakage measurements from the leaves (Janda *et al.,* 2000), application of SA enhanced photosynthetic rate (Khan *et al*., 2003), increased stomatal conductance and transpiration (Quiroz *et al.,* 2001 and Tari *et al.,* 2002) and it has been suggested that it directly involved in signaling various antioxidant responses (Larkindal and Knight, 2002).

Selenium (Se) is an essential micronutrient needed in antioxidantion and hormone balance in higher plants (Stadtman, 1992). Recently, Hartikainen *et al.* (2000) demonstrated that, it acts as antioxidant and can stimulate the plant growth. Selenium can increase the tolerance stress to Uv-induced oxidative stress (Hartikainen *et al.,* 2000, Xue and Hartikaninen, 2000). Application of Se up to 50 ppm in soybean increased the yield by preventing chlorophyll degradation and maintening large leaf area duration

(Djanaguiraman *et al.,* 2005). Moreover, Kong *et al*., (2005) studyed the effect of applying selenium to sorrel plants under saline stress, they reported an increase in antioxidant enzymes and furthermore an improvement in the integrity of plasma, mitochondrial and chloroplast membranes. The results of the above mentioned researches therefore suggesting an antioxidant function of Se, define this element as a strengthener of osmotic capacity for its role in the maintenance of cell memberanes. Recently it has been shown that selenium has the ability to regulate the water status of plant under drought conditions. Selenium causes enhancing water relation in wheat tissue (Kuznetsov *et al*., 2003).

Jasmonic acid (JA) as an endogenous growth regulator that play an important roles for regulating the stress response (Creelman & Mullet, 1997). The amelioration of chilling injury and osmotic stress by JA have also been reported on rice, peanut seedlings and cucumbers (lee *et al*., 1996 and Wang, 1999). Many researches indicated that JA significantly reduced chilling injury of mango (Gonzalez *et al*., 2000), tomato (Ding *et al*., 2001). Additionally, field applications of JA obviously decrease climaetric ethylene production of peach fruits at harvest by regulating activities of enzymes in cell wall metabolism (Ziosi *et al*., 2008) and reduce ion leakage in strawberry (Gonzalez *et al*., 2006).

In recent research, MeJA has been applied to reduce the development of chilling injury symptoms (Ding *et al.,* 2001, Fung *et al.,* 2004 and Cao *et al.,* 2007). It was observed that Me JA could be liked to oxidative stress and different effects of it on protective enzymes activities could associated with H_2O_2 metabolism (Wang *et al.,* 2005 and Ali *et al.,* 2006).

Chilling injury symptoms can be the consequence of oxidative stress from excess reactive oxygen species (ROS) that induce periodation and breakdown of unsaturated fatty acids in membrane lipids (Lyons, 1973). A previous study has shown that a positive relationship exists between the antioxidant enzyme activity and the chilling tolerance (Sala, 1998).

The objective of this study was to evaluate some pretreatments (chilling, selenium, salicylic acid and jasmonic acid) for cold stress protection beside stimulating fruit setting in order to obtain early yield in spring.

MATERIALS AND METHODS:

The experiments were carried out during the winter seasons of 2008 and 2009 at the experimental Farm of Shibin El-kom, Faculty of Agriculture, Minufiya University.

Super Strain-B cv. tomato seedlings were germinated under mist. The seedlings were transfered in a greenhouse under natural light until the seedling reached 15cm (average height) . The 15cm height seedlings were subjected to five treatments as followes:

1- The seedlings were exposed to cold pretreatment i.e. 10°C for 96 h. in the dark in the refrigerator. Seedlings were then transfered from the refrigerator

and placed in the greenhouse under natural conditions for one week for survival. Surviving seedlings were those recovered full turgidity within 48 h. after chilling, whereas non-surviving plants were those fully disicated and exhibited no evidence of resumed growth after one week.

Before transplanting, other seedlings treated with some pretreatments as following:

2- Jasmonic acid at 0.02 mM.

3- Salicylic acid at 0.1 mM.

4- Selenium at 0.6 mM. All seedlings were soaked for two hours before transplanting immediately.

5- The plants grown under the field conduction (cold stress) considered as a control plants.

The previous treatments used as spraying treatments on the vegetative growth at flowering stage, i.e., 4 and 6 weeks from transplanting. Tween 20 was added to the spraying solution at 0.5% as surfactant. Transplanting tomato seedlings were conducted on 18th and 20th of January in both seasons, respectively.

Temperatures were recorded continuously during field trials and the average temperature for day and night are presented in (Table 1).

The experimental design was a complete randomized block in three replicates. The plot area was 12 m^2 as it included three rows, 5 m long and 60 cm in width.

Fertilizers at the rate of 300 kg superphosphate was added at soil preparation, 250 kg ammonium sulphate and 150 kg potassium sulphate. N and K fertilizers were added in two equal parts, after 20 and 45 days from transplanting.

Four plants were taken randomly from each treatment at 75 and 90 days after transplanting and the following data were recorded:

1. Growth characters:

Recorded data includes plant height (cm), number of branches and leaves/plant, fresh leaves and stems and roots (g), and leaf area/plant (cm^2) according to (Roods and Blood-Worth, 1964). All parts of vegetative samples were separated and oven dried at 70°C for 72 hrs. to determine root and shoot dry weight per plant (g).

Table (1): Average temperature (°C) and relative humidity during experimental periods 2008 and 2009.

Temperature Values	December	January	February
Maximum Temp.			
2008	24.0	22.9	25.6
2009	23.0	20.9	24.9
Minimum Temp.			
2008	10.3	7.9	7.9
2009	9.4	7.0	9.1
Average Day Temp.			
2008	21.7	19.6	23.0
2009	20.1	18.9	23.8
Average Night Temp.			
2008	11.9	9.3	10.3
2009	10.8	8.9	9.1
Humidity (%)			
2008	62	65	65
2009	63	66	63
Soil Temp. (10cm depth)			
2008	18.1	15.2	16.1
2009	19.1	14.9	15.8

Net assimilation rate (NAR) was calculated as the following equation:

$$NAR = \frac{(W_2 - W_1)}{(L_2 - L_1)} \cdot \frac{(\log_e L_2 - \log_e L_1)}{(t_2 - t_1)} \quad (mg/cm^2/day)$$

Where : W_1 , W_2 **: The total dry weights per plant at** t_1 , t_2

L_1 , L_2 **: The leaf area per plant** cm^2 **at** t_1 , t_2

t_1 , t_2 **: Duration in days between the first and second samples**

At the second samples (flowering stage, 90 days from transplanting) the 3rd and 4th leaves from the top were collected and the following determinations were made in these samples:

2. Relative water content (RWC): It was measured as the method described by (Barrs and Weatherley, 1962).

3. Membrane leakage: It was determined following the method of (Leopold *et al.,* 1981).

4. Chemical analysis:

a) Photosynthetic pigments:

chl. *a* , *b* and carotenoids were estimated in fresh leaves as described by (Witham *et al.,* 1971) and expressed as mg/g dry weight.

b) Antioxidant Enzymes activity:

Peroxidase, phenoloxidase and catalase activity were measured in the fresh leaves using the methods described by Fehrman and Dimond

(1967), Broesh (1954) and Samantary (2002), respectively.

c) Proline concentration was measured in fresh leaves following the method of Bates *et al.,* (1973).

d) Total soluble sugars were estimated in dry leaves according to the method of Dubois *et al.,* (1956).

e) Total Amino Acids were estimated in dry leaves according to the method of Rosen (1957).

f) Mineral concentration: N, P and K were measured in dry leaves as described by A.O.A.C. (1990), Snell and Snell, (1954) and Chapman and Pratt (1961), respectively.

5. Flowering:

The flowers of six plants from each treatment were tagged to determine the early yield.

6. Yield and its components:

a) The early yield was the sum of fruit weight of the first three pickings.

b) Total yield was calculated from the first picking up to the last of harvesting.

c) Total soluble solids (TSS) percentage was determined in tomato fruits by using a hand Abb refractometer according to A.O.A.C. (1990).

d) Vit. C. content was determined (mg/100 ml of tomato juice) using the dye 2, 6 dichlorophenol indophynol as described in A.O.A.C. (1990).

All obtained data were subjected to statistical analysis with the help of Costat-c programe and the L.S.D. at 5% level was calculated according to Duncen's way.

RESULTS AND DISCUSSION

Growth analysis:

Salicylic acid application significantly promoted the shoot growth in terms of shoot length, No of branches, No of leaves, fresh weight of leaves, stems and roots (Tables, 2,3). The shoot length, No of branches and No of leaves were increased by 13, 129 and 25%, respectively in SA treatment compared with the control. Moreover Se promoted the previous parameters by 7, 59 and 16% respectively. Chilling and JA also significantly increasing the above mentioned parameters compared to control plants but not as SA and Se treatments. The same trend was recorded on leaves, stems and roots fresh and dry weight.

Leaf area and NAR were increased by 36% and 53% over control, respectively as affected by SA, followed by Se treatment. The protective effect of SA treatment against cold stress was shown by the greater root dry weight and root/shoot ratio (Table 3). Results could be explained as SA as a natural plant product combines growth enhancement and antisenscence properties (Raskin, 1995). SA increases plant height, growth, reverses ABA-induced stomatal closure and leaf abscission and stimulates adventitious root initiation (Rajasekaran and Blake 1999 and Kong and Saltveit 2002).

Table (2)

Table (3)

RWC and Cell membrane leakage

RWC was lower in plants grown under cold conditions. Treating plants with SA and Se increase the water content of plants compared to untreated plants (Table 4). JA and chilling treatments recorded the same effect in increasing RWC compared to control plants. Szepesi *et al.,* (2005) show that salicylic acid treatment reduced K^+content in leaves and increased water content. Plants treated with SA and Se prevented the decrease in biomass caused by cold stress by increasing root biomass, water content. These results are in agreement with Hu *et al.,* (2002) and Deef , (2007).

Meanwhile, chilling and JA treatments had non significant effect on root dry matter as well as root/shoot ratio and slightly increased RWC (Meng *et al.,* 2009 and Germ & Stibilj, 2007).

The more interested data showed that SA and Se were higher protectant to membrane stability under cold stress, where was the higher membrane leakage or damage recorded under cold stress followed by JA and chilling treatments (Table 4). Also, (Gonzalez *et al.,* 2006 and Meng *et al.,* 2009) investigated the effects of JA treatment on alleviating chilling injury and metabolism of cell wall of peach fruits under low temperature.

Jasmonic acid treatmet could protect cell membrane by decreasing

membrane-lipid peroxidation and maintaining high superoxide dismutase (SOA) activity in strawberry under water stress (Wang, 2005) and reduce ion leakage (Gonzalez *et al.,* 2006)

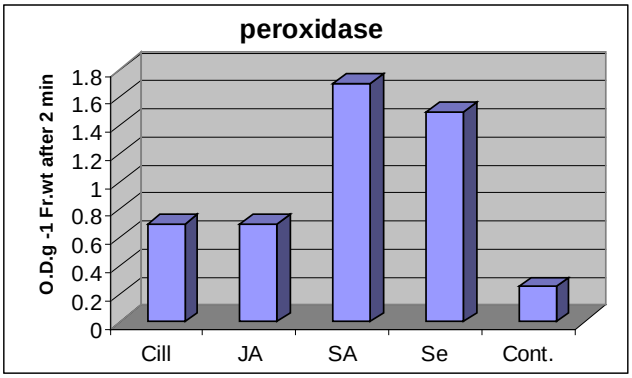
Chlorophyll concentration

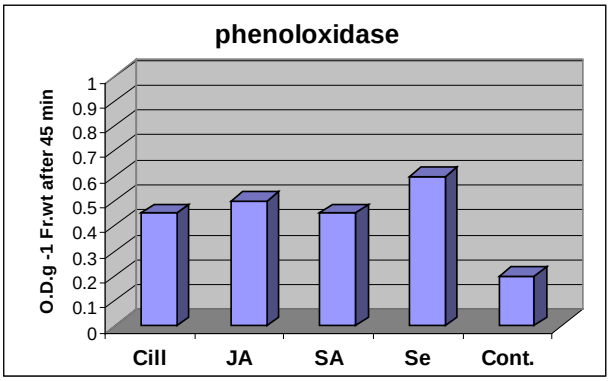
Tomato plants treated with SA prevented the decrease in chlorophyll and carotenoids caused by cold stress (Table 4). Since SA improved the photosynthetic performance of plants under cold stress conditions (Ananieva *et al.,* 2004) and chlorophyll a fluorescence as recorded by Deef (2007) could give insight into the ability of plant to tolerate environment stresses. Moreover Se treatment recorded the same effect of SA in induction of photosynthetic pigments compared to control and chilling treated plants. These results are in agreement with those Germ (2008) on potato. The increased chlorophyll content in SA and Se treated plants might attributed to the efficient scavenging of ROS by oxidase and peroxidase activities or otherwise they would have destroyed the chlorophyll pigments (Thomas *et al.,* 2001 and Djanaguiraman *et al.,* 2005).

Table (4)

Enzymes activity

SA and Se treated tomato plants under low temperature condition increased catalase activity in leaves. Moreover the other enzymes associated with the antioxidants defense peroxidase and phenoloxidase also significantly increased compared to untreated plants (Fig 1). Moreover chilling and JA treatments significantly increased activity of previous enzymes compared to untreated plants but not as SA and Se treatments. Moran *et al.,* (1994) and loggini *et al.,* (1999) reported that, increasing the activity of antioxidants enzymes may play a role in maintaining low levels of hydrogen peroxide in the cell. Hydrogen peroxide, even, at low concentration, inhibits chloroplast sulfhydryl containing enzymes by readily oxidizing their sulfhydryl groups. Therefore, it is important for plant cells to keep level of hydrogen peroxide low or to scavenge it efficiently. For that using SA and Se as foliar application under cold stress may be prevent plant against hydrogen peroxide formation by increasing antioxidants enzymes which protect cell membrane from damage (Germ and Stibilj, 2007 and Deef, 2007). Beside that JA and chilling treatments significantly increased phenoloxidase activity which protect plants against cold stress and produced good quality fruits (Meng *et al.,* 2009). Under stress conditions, some soluble peroxidase isoforms can be released from the cell wall and circulated inside the apoplast of the intact plant. Thus peroxidase protects cell against the damaging effects of H_2O_2 during an oxidative - burst response (Bolwell *et al*., 2002). Only one work about the induction of peroxidase and by exogenous JA was reported by (Garrido *et al*., 2003), stimulating the O_2 synthesis by root cells. These results are in agreement with the finding of Shim *et al*., (2003).





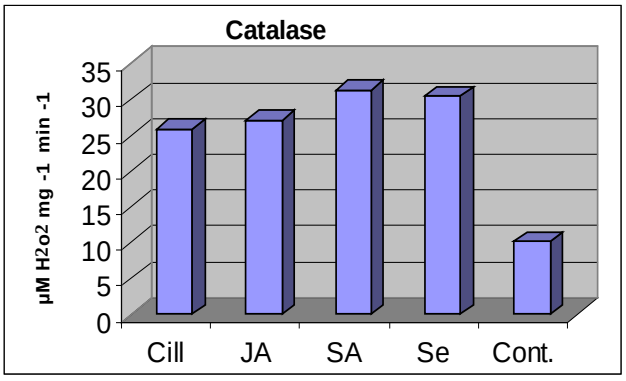


Fig (1): Effect of chilling hardening, Jasmonic acid and antioxidantson peroxidase, phenoloxidase and catalase activities in leaves of tomato plants during 2008 season.

Compatible Osmolytes (Proline, TS, TAA and K^+)

In the presence of SA, leaves accumulated different compatible osmolytes such as sugars, sugar alcohol, proline and K^+ (Tari *et al.,* 2002, and Deef, 2007). In the present work, SA and Se foliar application on tomato plants under cold stress condition significant increased proline by 8.7% and 80% compared with control plants, respectively in the first season (Table 5). Moreover, the increase in TS was 28% and 21% and in TAA was 64 and 41%, respectively in the first season (Table 5). Meanwhile Se was more effective in increasing K^+ than SA under cold condition, in both seasons (Table 6).

Obtained results are in accordance with those of Deef (2007) who cited that, possibly in seedlings pre- treated with SA and subjected to stress, where growth is greater and plant status better, damage is less by a significant increase in total sugars, crude protein and total carotenoids content of yellow maize grains (Farooq *et al.,* 2009).

It can be noted that also JA and chilling pre-treatment significantly increased osmolytes compatible solutes, proline, TS and TAA compared to untreated plants but not as SA and Se treatments.

Nitrogen and phosphorus minerals

The present data in (Table 6) showed that foliar application of SA and Se under cold condition significantly increased N and P compared to control plants. Moreover, chilling and JA followed the previous treatments in increasing N and P compared to control plants. These results are in agreement with the founding of (Yildirim *et al.,* 2008).

Yield and its components

Early yield recorded a highly significant increase when tomato plants treated by SA (66%), Se (64%), JA (60%) and chilling (38%) compared to control plants in the first season. The same trend was observed in total yield in the second season (Table 7).

All treatments under studying significantly increased tomato fruits quality in terms of vitamin-C and TSS compared to those of control plants.

The data suggest that SA and Se play an important antioxidant role in protecting tomato plants from cold stress condition. SA and Se are direct scavenge of hydroxyl radicals (Tunhui *et al.,* 2009).

Data have been presented suggesting a salicylic-iron complex, with SOD activity catalyzing dismutation of superoxide radicals (Jay *et al.,* 1999). Therefore, treating tomato plants with SA and Se, may act directly as a preformed antioxidant to scavenge ROS and/or indirectly modify the redox balance by activating antioxidant responses as suggested by (Yang *et al.,* 2004) for rice plants, and (Krantev *et al.,* 2008) in maize plants. The growth stimulating effect of SA and Se may be related to its antioxidative

Table (5)

Table (6)

Table (7)

function as demonstrated by diminished lipid peroxidation, H_2O_2 and superoxide radical production and higher content of chlorophyll a, b and total chlorophyll than control, which reflected higher yield with good quality. This finding is in accordance with (Tunhui *et al.,* 2009) and (Yildirim *et al.,* 2008).

Hence the present study clearly indicate the antioxidative role of Se by enhanced antioxidant enzymes production which in turn postpones the senescence phenomenon in tomato grown in winter season. Also, these findings suggest that SA treatments can ameliorate the negative effect of low temperature on the growth with early and high tomato yield.

In conclusion, cold stress severely hampers the tomato performance stimulate synthesis of compatible solutes and activation of antioxidant system by SA, Se and JA application improved the integrity of cellular membranes and enabled the tomato plant to maintain tissues water status and as a result photosynthesis, antioxidants enzymes and general metabolism which reflected higher and good quality of tomato yield.

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حماية نباتات الطماطم ضد البرودة باستخدام مضادات الأكسدة والتقسية بالبرودة وحمض الجسمونيك

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الملخص العربي

أجريت تجربتان حقليتان خلال شتاء موسمى ٢٠٠٨ ، ٢٠٠٩ لدراسة تأثير بعض المعاملات مثل التقسية بالبرودة وحمض الجسمونيك وحمض السلسليك والسلينيوم على مقاومة نباتات الطماطم للبرودة ، وكانت الدراسة لتأثير المعاملات سابقة الذكر على النمو والمحتوى المائى النسبى للأوراق ومرونة الجدر الخلوية وبعض الصفات الكيمائية والتزهير وكذلك المحصول وجودته .

. وقد وجد أن جميع المعاملات تحت الدراسة أدت إلي زيادة معنوية فى طول النباتات وعدد الأفرع وعدد الأوراق والوزن الغض والجاف لكل من الأوراق والسيقان والجذور، كما أنها أدت إلى زيادة المساحة الورقية وكفاءة التمثيل الضوئى مقارنة بالنباتات غير المعاملة(الكنترول) .

. إزداد معنويا المحتوى المائى النسبى وكلورفيل (أ ، ب) و الكلوروفيل الكلي(أ + ب) والمركبات الأسموزية مثل البرولين والسكريات الكلية والأحماض الأمينية الكلية وكذلك البوتاسيوم بجميع المعاملات تحت الدراسة ، بينما أدت جميع المعاملات إلي حدوث نقصا معنويا فى تلف الجدر الخلوية بالمقارنة بالكنترول .

ـ أدت جميع المعاملات السابقة إلي حدوث زيادة معنوية فى محتوى الأوراق من النيتروجين والفوسفور مقارنة بالكنترول . و كذلك إلى زيادة معنوية فى المحصول المبكر والمحصول الكلى وجودته مثل محتوي عصير الثمار من فيتامين (ج) و محتوى الثمار من المواد الصلبة الكلية و السكريات الكلية وذلك مقارنة بالكنترول.

. كانت أفضل المعاملات هى المعاملة بحمض السلسليك يليها السلينيوم بالمقارنة بمعاملة التقسية

بالبرودة وحمض الجسمونيك وهذا مقارنة بنباتات الكنترول .

تقترح نتائج هذه الدراسة معاملة نباتات الطماطم بالسلينيوم او حمض السلسليك حيث أنها تلعب دوراً هاما كمضادات للأكسدة والتى يمكنها حماية نباتات الطماطم من البرودة . حيث تعطي نباتات الطماطم نمو جيد و محصول مبكر مرتفع و ثمار ذات جودة عالية.

Table (2): Effect of chilling hardening, Jasmonic acid and antioxidants on some vegetative growth characters of tomato plant during 2008 and 2009 seasons.

Seasons	2008						2009					
Characters / Treatments	Plant height (cm)	No.of branches /plant	No. of leaves /plant	Leaves F.wt. (g/plant)	Stem F.wt. (g/plant)	Roots F.wt. (g/plant)	Plant height (cm)	No.of branches /plant	No. of leaves /plant	Leaves F.wt. (g/plant)	Stem F.wt. (g/plant)	Roots F.wt. (g/plant)
Chill.	91 c	7.67 c	36.0 c	135.26 c	166.22 d	27.24 d	87 d	8.67 c	36.5 c	153.31 c	202.31 d	35.44 c
JA	95 b	8.67 bc	39.0 b	137.22 c	194.23 c	38.28 b	91 c	9.00 c	39.5 b	145.31 d	232.24 c	34.22 c
SA	102 a	13.00 a	42.6 a	217.24 a	239.22 a	40.31 a	108 a	14.00 a	43.1 a	212.48 a	400.10 a	53.22 a
Se	96 b	9.00 b	39.8 b	164.25 b	227.14 b	35.71 c	96 b	10.00 b	40.4 b	160.15 b	244.25 b	40.26 b
Cont.	90 c	5.67 d	34.2 d	126.24 d	141.28 e	21.56 e	86 d	6.00 d	34.8 d	132.20 e	159.38 e	25.48 d

Table (3): Effect of chilling hardening, Jasmonic acid and antioxidants on some vegetative growth characters of tomato plant during 2008 and 2009 seasons.

Seasons	2008							2009						
Characters / Treatments	Leaves D.wt. g/plant	Stem D.wt. g/plant	Shoot D.wt. g/plant	Roots D.wt. g/plant	root / shoot ratio	LA/ plant (cm^2)	NAR (mg/ cm^2/ day)	Leaves D.wt. g/plant	Stem D.wt. g/plant	Shoot D.wt. g/plant	Roots D.wt. g/plant	root / shoot ratio	LA/ plant (cm^2)	NAR (mg/ cm^2/ day)
Chill.	30.45 c	25.19 c	55.64c	8.18 b	0.147d	2953 c	0.299 c	30.28 c	27.35 b	57.63d	9.18 b	0.159d	2968c	0.231c
JA	31.25 bc	26.39 c	57.64c	9.20 b	0.160b	3266 b	0.324 b	32.22 bc	28.25 b	60.47c	10.17 b	0.160 d	3286b	0.328b
SA	39.16 a	35.45 a	74.61a	11.23 a	0.151c	3473 a	0.373 a	36.47 a	42.20 a	78.67a	13.27 a	0.169c	3489a	0.384a
Se	32.16 b	28.29 b	60.45b	10.31 a	0.171a	3269 b	0.334 b	34.30 ab	30.35 b	64.65b	12.18 a	0.188a	3273b	0.354a
Cont.	30.45 c	21.36 d	51.81d	8.19 b	0.158c	2558 d	0.243 d	29.53 c	22.30 c	51.83e	9.34 b	0.180b	2561d	0.246d

Table (4): Effect of chilling hardening, Jasmonic acid and antioxidants on relative water content (RWC), membrane leakage (ML) and photosynthetic pigments (chl. a,b and carotenoids) of tomato leaves during 2008 and 2009 seasons.

Seasons	2008						2009					
Characters / Treatments	RWC %	ML %	Chl. a mg/g D.Wt	Chl. b mg/g D.Wt	Chl. a+b mg/g D.Wt	Carot. mg/g D.wt	RWC %	ML %	Chl. a mg/g D.Wt	Chl. b mg/g D.Wt	Chl. a+b mg/g D.Wt	Carot. mg/g D.wt
Chill.	80.3 d	47 b	3.48 b	1.71 b	5.19 c	2.25c	81.2 d	45 b	3.51 b	1.82 b	5.33 b	2.35c
JA	82.2 c	40 c	3.99 b	1.90 b	5.89 b	2.22c	83.5 c	42 b	4.10 a	1.82 b	5.92 b	2.28c
SA	87.5 a	30 e	4.81 a	2.41 a	7.22 a	2.51b	88.1 a	31 c	4.50 a	2.64 a	7.14 a	2.60b
Se	85.1b	35 d	4.24 a	2.13 a	6.37 b	3.48a	86.1 b	33 c	4.31 a	2.22 a	6.53 a	3.51a
Cont.	78.8e	70 a	2.09 c	1.41 c	3.40 d	2.06d	78.1 e	75 a	2.10 c	1.31 c	3.51 c	2.12d

Table (5): Effect of chilling hardening, Jasmonic acid and antioxidants on some enzymes activity, proline, total sugars and total amino acids in leaves of tomato plant during 2008 and 2009 seasons.

Seasons	2008						2009					
Characters / Treatments	Peroxi-dase O.D.g^{-1} Fr.wt after 2 min	Phenolo xidas O.D.g^{-1} Fr.wt after 45 min	Catalas e µM H_2O_2 mg^{-1} min^{-1}	Proline µg/g D.Wt	Total sugars mg/g D.Wt	Total amino acids mg/g Dr.wt	Peroxi-dase O.D.g^{-1} Fr.wt after 2 min	Phenolo xidas O.D.g^{-1} Fr.wt after 45 min	Catalas e µM H_2O_2 mg^{-1} min^{-1}	Proline µg/g D.Wt	Total sugars mg/g D.Wt	Total amino acids mg/g Dr.wt
Chill.	0.70 c	0.45 b	25.9c	1050 d	8.91 c	27.1c	0.60 d	0.45 b	26.1b	810 c	8.73 c	26.9d
JA	0.70 c	0.50 b	27.2b	1250 c	9.21b	28.8c	0.90 c	0.44 b	27.5b	1210 b	9.53b	29.1c
SA	1.70 a	0.45 b	31.4a	1400 a	10.82a	39.2a	1.80 a	0.50 a	31.8a	1325 a	10.91a	39.8a
Se	1.50 b	0.60 a	30.5a	1350 b	10.25 a	33.8b	1.20 b	0.60 a	30.9a	1375 a	10.16 a	34.1b
Cont.	0.25 d	0.20 c	10.2d	750 e	8.45 c	23.9d	0.25 e	0.35 c	23.5c	740 d	8.25 c	22.1e

Table (6): Effect of chilling hardening, Jasmonic acid and antioxidants on some minerals concentration in leaves of tomato plant during 2008 and 2009 seasons.

Seasons	2008			2009		
Characters / Treatments	N g/100 g D.Wt	P g/100g D.Wt	K g/100gD.Wt	N g/100 g D.Wt	P g/100gD.Wt	K g/100g D.Wt
Chill.	3.20 b	1.55 b	5.52 c	3.10 c	1.45 b	5.71 c
JA	3.40 b	1.22 b	6.24 b	3.50 b	1.22 b	6.63 b
SA	4.80 a	1.78 a	5.94 c	5.36 a	1.65 a	6.04 b
Se	4.00 a	1.60 a	7.27 a	3.60 b	1.50 a	7.07 a
Cont.	2.80 c	0.95 c	4.62 d	2.60 d	0.79 c	4.54 d

Table (7): Effect of chilling hardening, Jasmonic acid and antioxidants on total yield, early yield and its components of tomato plant during 2008 and 2009 seasons.

Seasons	2008				2009			
Characters / Treatments	Total yield ton/fed	Early yield ton/fed	Vit. C mg/100 ml juice	T.S.S	Total yield ton/fed	Early yield ton/fed	Vit. C mg/100 ml juice	T.S.S
Chill.	20.05 c	10.02 c	0.34 c	5.4 a	21.00 c	11.10 c	0.36 b	5.2 a
JA	21.82 b	11.64 b	0.37 b	5.2 a	22.50 b	12.30 b	0.41 a	4.5 b
SA	23.45 a	12.11 a	0.39 a	5.6 a	24.28 a	13.20 a	0.43 a	5.5 a
Se	23.20 a	12.01 a	0.37 b	5.8 a	24.08 a	13.01 a	0.41 a	5.6 a
Cont.	15.03 d	7.28 d	0.28 d	4.2 b	17.50 d	8.38 d	0.30 c	4.3 b