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Chilling Tolerance Enhancement in Tomato (*Solanum lycopersicum*) By Exogenous Application of Aspirin

Salwa A. Orabi[1], S.R. Salman[2], S.D. Abou-Hussein[2] and W.A. El-Tohamy[2]

[1]Botany Dept., and [2]Vegetable Research Dept., Agricultural & Biological Research Institute, National Research Centre (NRC), 33 El-Buhouth St., 12622 Dokki, Giza, Egypt.

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ABSTRACT

Two pot experiments were conducted during two growing seasons 2017/2018 and 2018/2019 at the green house of the National Research Centre, Dokki, Giza, Egypt. To improve cold tolerance, acetyl salicylic acid (aspirin) (1, 2 mM) was investigated. The work focused on study the effect of aspirin (ASA) on growth criteria (plant height, number of leaves, fresh and dry weights of leaves and root of tomato/plant), total soluble Protein, total chlorophyll, antioxidant enzyme (APX, GR, Gly1) activities and specific activities, super oxide (O_2^{-}), electrolyte leakage, and yield. Obtained results revealed that plants grown under low temperature and foliarly treated after transplanting with ASA at the concentration of 2mM followed by 1mM mitigated the harmful effects of low temperature stress through the enhancement of their protective parameters, such as antioxidant enzymes activity, total soluble protein and total chlorophyll. ASA at 2mM recorded the highest increments in growth criteria, APX, GR, GlyI activities and specific activities, total soluble protein, total chlorophyll and total soluble solids. Remarkable decreases were also obtained in O_2^{-} and electrolyte leakage (EL) with the used concentration of aspirin treatments. The results also, showed that the highest value of yield per plant was recorded with plants received aspirin at the concentration of 2mM. Based on the obtained results, it could be suggested that the protection mechanism had helped the plants to increase their resistance against chilling stress, through mainly the decrease in membrane damage symptoms leading to intercellular osmotic adjustment.

Keywords: Tomato, Low temperature, Antioxidant enzymes, Electrolyte leakage, ROS

1. Introduction

Low temperature (LT) is considered to be one of the major abiotic stresses, it causes physiological and metabolic disorder leading to reduction of growth and productivity, reduced stomatal conductance, photosynthetic efficiency, changes in protein structure and enzyme activities (Prasad *et al.,* 1994). Under LT stress there will be inhibition of photochemistry efficiency and the normal mitochondria electron transport might be disrupt, causing the production of reactive oxygen species (ROS) (Purvis *et al*., 1995; Orabi, 2004; Orabi *et al.,* 2010, 2017[a], 2018 and Abd El-Razek *et al.,* 2019) which may include singlet oxygen (1O2), superoxide anion (O2,´), hydrogen peroxide (H2O2), and hydroxyl radical (OH,) (Gill and Tuteja, 2010). If the plants are to be cultivated in December, January and February, they will be subjected to low temperature. Minimum level of night temperature during these months drops several times below 10°C. Under prolonged stress excess levels of ROS are produced and caused cell membrane lipid peroxidation (Orabi, 2004; Ahmed *et al.,*2009,2010; Hussein and Orabi, 2008; Hussein *et al.,* 2009; Mekki *et al.,* 2010 and Abd El-Motty and Orabi, 2013) beside protein denaturation, carbohydrate oxidation, photosynthetic pigment breakdown, impaired enzyme activity, damage to nucleic acids and programmed cell death (Bose *et al.,*2014). Therefore, plants need a delicate balancebetween ROS generation and scavenging (Mekki and Orabi, 2007; Orabi and Mekki, 2008; Kassab *etal.,* 2012;

Corresponding Author: Salwa A. Orabi, Botany Dept., Agricultural & Biological Research Institute, National Research Centre (NRC), 33 El-Buhouth St., 12622 Dokki, Giza, Egypt.
E-mail: dr.salwaorabi@yahoo.com

Orabi *et al.,* 2016 , 2017[b,c]).

Tomato (*Lycopersicon esculentum* L.) is a member of the Solanaceae family, it is considered to be one of the most important vegetables grown in Egypt. It is used as a fresh vegetable beside its importance as a raw material for agricultural industry. In addition it is a rich source of lycopene, vitamins and minerals. Lycopene is responsible for the characteristic deep red color of ripe tomato fruits and tomato products (Helyes *et al.,* 2009). Lycopene may help to counteract the harmful effects of substances called "free radicals" and different types of cancer, it is a key intermediate in the biosynthesis of many important carotenoids, such as beta-carotene and xanthophylls (DeStefani *et al.,* 2000). Tomato plants after ecxposure to low temperature will suffer from chilling injury as a result of exposure to low, but non-freezing temperatures (ca. >10˚C) (Raison and Lyons, 1986).

Phytohormones are natural plant growth regulators (PGRs) that act as signaling molecules and are present in plants at very low concentrations. They are key regulators of complex root to shoot interactions that control plant growth and development. Aspirin, a trade name for acetylsalicylic acid (ASA). It is known that in aqueous solutions, ASA is hydrolyzed almost entirely to SA, which is active ingredient (Mitchell and Broadhead, 1967). Despite the fact that aspirin was not identified as a natural product, it is widely used by many scientists in their experiments according to the similarity of their physiological effects. However, Salicylic acid is not the same thing as aspirin Salicylic acid is an endogenous growth regulator with phenolic nature, which participates in regulation of several physiological processes in plants, such as stomatal closure, ion uptake, inhibition of ethylene biosynthesis and transpiration (Khan *et al.,* 2003; Shakirova *et al.,* 2003). These signaling molecules can play significant roles in plants' responses to biotic and abiotic stresses (Peleg and Blumwald, 2011).

Application of ASA or SA induced tolerance in plants to many biotic and abiotic stresses including fungi, bacteria and viruses, chilling, salinity, drought and heat (Orabi *et al*., 2010,2013, 2018; El-Tohamy *et al*., 2020 and Khan *et al*., 2010).

2. Materials and Methods

2.1. Experimental procedure

Two pot experiments were conducted during two successive winter seasons (2017-2018) and (2018-2019) at wire house of the National Research Centre, Dokki, Giza, Egypt, to study the effect of two levels of ASA (1mM and 2 mM) on growth, biochemical criteria and fruit yield quantity and quality of two tomato cultivars (Strain B and Florida) grown under low temperature conditions in sand-ponic culture. Seedlings (one true leaf stage) were transplanted carefully in pots filled with washed sand (2 seedlings /pot) at first week of December during two successive seasons. During this experiment, plants are subjected to low temperature where level of night temperature drops several times below 10˚C. The plants were supplied with nutrient solution via irrigation. The nutrient solution contains all necessary elements that required for plant growth. The base solution contained (mg L^{-1}): 200 potassium, 200 nitrogen, 200 calcium, 54 phosphorus, 64 sulfur, 49 magnesium, 5 iron, 0.5 boron, 0.5 manganese, 0.05 zinc, 0.02 copper and 0.01 molybdenum. Electrical conductivity (EC) and pH were measured and adjusted regularly. Tape water used had: pH of 7.5 and EC of 0.35 dSm^{-1}. The pH of the nutrient solution was measured using a portable pH meter, H1 9023 (Hanna Instruments, Padova, Italy). The experiment was arranged in a complete randomized design with 15replicates for each treatment. The treatments were two levels of ASA (1 mM and 2 mM) which applied exogenously on plants after 15 and 30 days from transplanting.

2.2. Data recorded

Random samples of plants were collected at 75 days after transplanting from each treatment to determine some growth parameters (plant height, leaves number / plant, fresh and dry weights of leaves and root/plant) as well as estimate antioxidant enzymes, super oxide (O^-_2) , electrolyte leakage (EL), total soluble protein and total cholorophyll (SPAD values), O^-_2 scavenging activity . At harvest, tomato fruits were collected weekly and total yield was calculated as g/plant. Fruit quality i.e. total soluble solids (TSS) of fruit juice was determined.

2.3. Biochemical Constituents measurements:

2.3.1. Enzymatic and non enzymatic antioxidants measurements:

For enzyme determination**:** The method used for extracting the enzyme was that of Mukherjee and Choudhuri(1983). The activity and specific activity of APX (EC1.11.1.11) was determined according to Nakano and Asada (1981). One unit of APX was defined as the amount of enzyme that breaks down 1µ mol of ascorbate per min. GR activity and specific activity (EC 1.6.4.2) was determined according to Zanetti [30]. One unit of GR was defined as the amount of enzyme that decreases 1A340 per min. Glyoxalase I (Gly I, EC: 4.4.1.5) activity and specific activity was measured following Hasanuzzaman *et al.,* (2011). Soluble protein was determined according to Bradford (1976). Total Chlorophyll was determined using chlorophyll meter (Model: TYS-A, Zhe Jang Top Instrument Co. LTD., Hangzhou, China. Total soluble solids (TSS) were determined using a portable refractometer (Brixstix BX 100 Hs; Techniquip Corporation, Livermore, CA).

2.3.2. Oxidative damage measurements and scavenging

Determination of the generated superoxide redical ($O^{-}2$) was based on the reduction of the nitro blue tetrazolium (NBT) accordant to the method described by Doke (1983). Electrolyte leakage (EL) was measured using an electrical conductivity meter (Hanna Instruments, Bedfordshine, England) as described by Goncalves *et al*. (2007). Superoxide radical ($O^{-}2$) scavenging activity was determined as described by Beauchamp and Fridovich (1971) and modified by Ibrahim *et al*. (2013) .

2.4. Statistical Analysis:

The data obtained were subjected to standard analysis of variance procedure according to Snedecor and Cochran (1980). The values of L.S.D. were calculated whenever F values were significant at 5% level.

3.Results and Discussion

3.1. Effect on vegetative growth:

Data recorded on vegetative growth traits *i.e.* plant height, leaves number /plant, fresh and dry weights of leaves and root /plant as affected by Aspirin. Florida cultivar was characterized by higher significant increases in these growth parameters than those of Strain B cultivar under all treatments (Table 1). ASA at 2mM caused the highest significant increases in the growth parameters of the two tomato cultivars followed by 1mM. The stimulation effect of ASA on plant growth was confirmed by Abd El-Wahed *et al.* (2006) on yellow maize plants, Emongor, (2007) on cowpea plants and Martín-Mex *et al.* (2005) on African violet plant, El-Shraiy and Hegazi (2009) On pea and Kabiri and Naghizadeh, (2015). On barley. The increases in fresh and dry matter of stressed plants in response to ASA might be related to the induction of antioxidant response that increased the tolerance of plants to damage (Gunes *et al*., 2005).

This stimulative effect of Aspirin on tomato of the two cultivars might be resulted from its stimulatory effect on photosynthesizing cells (Zhoi *et al.,* 1999). This promotive role of Aspirin might be resulted from its bioregulator roles on various biochemical & physiological processes in plant such as regulation of sink / source, cell elongation, division, and differentiation, biosynthesis of proteins, activities of different enzymes, and increase in antioxidant capacity of plants (Blokhina *et al*., 2003).

Table 1: Effect of aspirin on some growth parameters of two tomato cultivars grown under low temperature conditions

Treatments	Plant height (cm)		Number of leaves		Leaves fresh weight (g)	
	Strain B cv.	**Florida cv.**	**Strain B cv.**	**Florida cv.**	**Strain B cv.**	**Florida cv.**
Control	45.50	55.00	18.00	23.33	45.11	60.82
ASA (1mM)	51.33	59.67	24.67	31.00	52.00	64.00
ASA (2mM)	57.00	62.33	28.67	34.33	57.13	73.26
L.S.D 5%	**3.78**		**2.92**		**5.35**	

Table 1: cont.

Treatments	Leaves dry weight (g)		Root fresh weight (g)		Root dry weight (g)	
	Strain B cv.	**Florida cv.**	**Strain B cv.**	**Florida cv.**	**Strain B cv.**	**Florida cv.**
Control	6.40	8.04	19.08	22.08	7.11	8.89
ASA (1mM)	7.90	9.31	20.66	26.78	8.97	10.22
ASA (2mM)	8.60	10.65	24.92	28.94	11.06	12.60
L.S.D 5%	**1.08**		**3.00**		**1.19**	

3.2. Effect on biochemical traits:

Florida cultivar was characterized by higher activities and specific activities of APX, GR and Gly I than Strain B cultivar either in treated or untreated plants (Fig 1). All ASA treatments mostly led to significant increases in all studied antioxidant enzymes (APX, GR & Gly I). Whereas, significant decrements were obtained in O_2^- and EL as a result of ASA treatments.). Many studies suggest the predominant role of the aspirin or salicylic acid in the modulation of the response of plants towards abiotic and biotic stresses by induction of the antioxidant ability (Orabi *et al.,* 2010 and Boukraa *et al*., 2014). In this regard, Ahmad *et al*. (2012) and Abd Elhamid *et al.* (2016) stated that at suboptimal condition of low temperature, priming maize or wheat seeds with SA or ASA induced activities of scavenging enzymes and increased the chilling tolerance.

Exogenous SA as one of the phytohormonse could regulate the synthesis and activities of antioxidant enzymes and increase plant tolerance to biotic and abiotic stress (He *et al*., 2002; Fayez and Bazaid, 2014). SA was found to enhance the activities of antioxidant enzymes such as POD when sprayed exogenously to the drought stressed plants (Hayat *et al*., 2010) or to the salinity stressed plants (Szepesi *et al*., 2008), might be due to its regulatory role at transcriptional and/or translational levels (Hayat *et al.,* 2005). In Brassica juncea, Yusuf *et al.* (2012) reported that SA enhanced the level of antioxidant system (SOD and POD) under stress and stress-free conditions. On the other hand, SA has the ability to inhibit the activity of CAT that lead to rise in the level of the H_2O_2 in vivo and stimulate the defense genes (Boukraa *et al.,* 2014).

APXs enzymes involved in scavenging H_2O_2 in water-water and AsA-GSH cycles using AsA as substrate, to catalyz the transfer of electrons from AsA to H_2O_2, to produc DHA and water (Pang and Wang, 2010). CR is one of the vital components of ASA – Gsh pathways, is primarily responsible for the regeneration of GSH from GSSG using NADPH as a reducing equivalent. This enzyme plays an important role by maintaining the reduced status of GSH and ASA pools and proper GSH/GSSG ratio that is more decisive in determining plant resistance to abiotic and biotic stresses than in the actual GSH content (Hossain *et al*., 2010). The elevated level of GR might increase the ratio of NADP+ to accept electron transport chin. Under these circumstances; the rate of electron flow to O_2 is reduced and this reduced the formation of (O^-_2) and the metal catalyzed formayion of OH, through the Haber- Weiss reaction (Hossain *et al*., 2013). Gly I, participates to catalyze the detoxification reaction of MG to D-lactate. Gly I converts MG to SLG utilizing GSH (Yadav *et al.,* 2005).

These increases might be attributed to the Aspirin direct function in enhancing active oxygen species (AOS) such as O^-_2 and H_2O_2 So the increases in antioxidant enzymes activities increases for decomposing the harmful AOS (Wendehenne *et al*., 1998). Similar results were obtained by Orabi *et al*. (2010) on cucumber plant, Orabi *et al*., (2013, 2015) on faba bean & tomato, Orabi *et al*., (2018) on mandarin fruits and Gholamnezhad *et al*. (2016) on wheat plant.

Under environmental stresses including LT stress reduction of chl biosynthesis and rapid degradation of chl are common occurrence (Orabi, 2004; Orabi *et al*., 2010; Mohanty *et al.,* 2006). Treatment of ASA as shown in (Fig 1) resulted in significant increase in chl of the two tomato cultivars. Similarly, exogenous application of SA significantly enhanced chlorophyll, net photosynthetic rate which could be due to improving the functional state of the photosynthetic machinery in plants either by the mobilization of internal tissue nitrate or by chlorophyll biosynthesis (Shi *et al.,* 2006). Suitable concentrations of salicylic acid inhibit chlorophyll degradation and increase photosynthesis by inhibition of chlorophyll oxidase enzyme activity (Belkhadi *et al*., 2010).

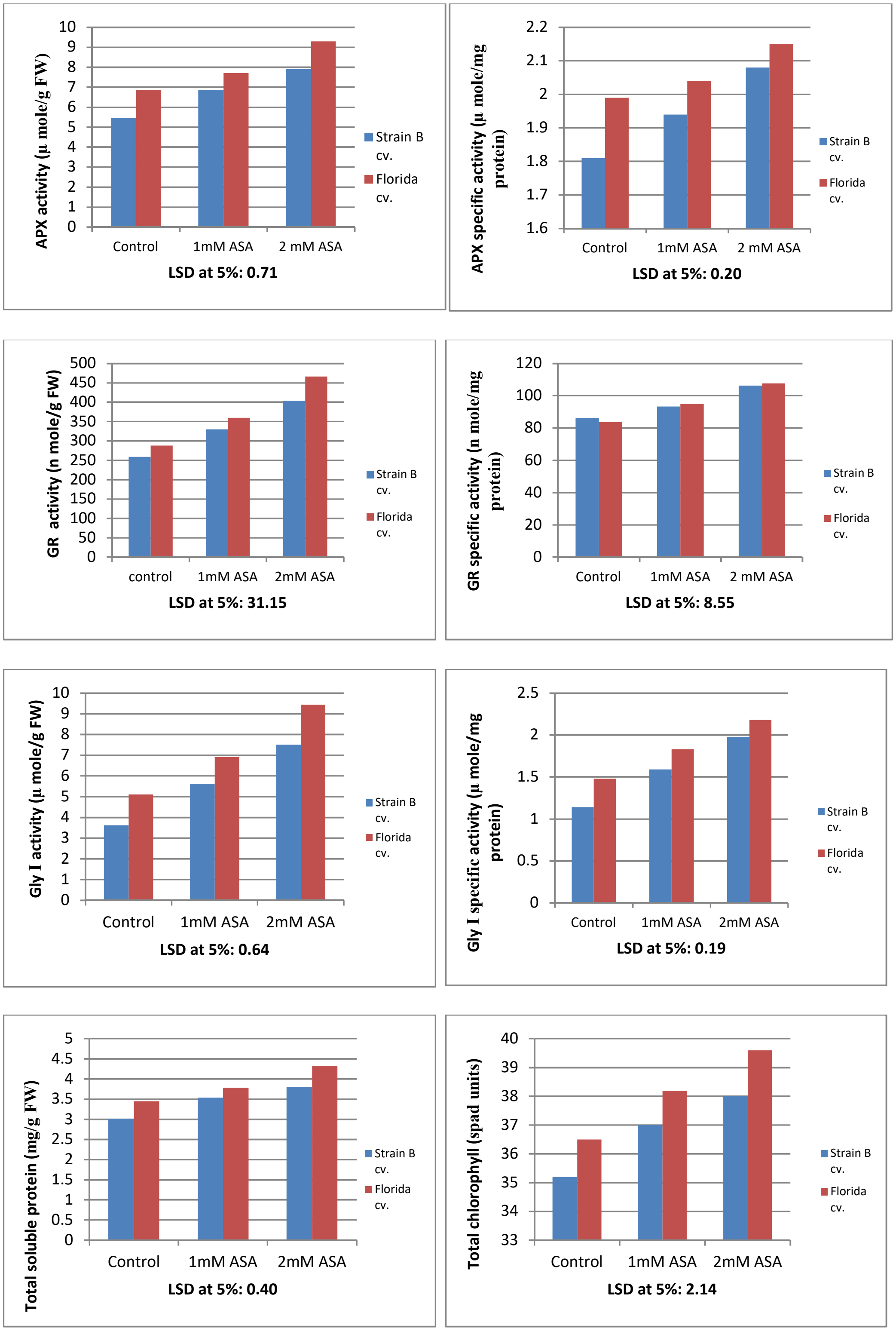


Fig 1: Effect of aspirin on some antioxidant enzyme activities and specific activities, total soluble protein and total chlorophyll of two tomato cultivars grown under low temperature conditions

Application of ASA recorded higher significant level in chlorophyll concentration (El-shrai and Hegazy, 2009). Moreover, Türkyýlmaz *et al*., (2005) suggested that, foliar spray with salicylic acid increased Chl a, Chl b and other photosynthetic pigments in bean plants under normal field conditions. The stimulation effect of ASA on chlorophyll concentration was confirmed by Zhao *et al*. (1995) on soybean.

Treatment of ASA gave significant increase in total soluble protein in the two tomato cultivars (fig 1). Similarly El-shrai and Hegazy (2009) on pea, Abd El-Wahed *et al.* (2006) on yellow maize and Vardhini and Seeta (1998) on peanut seed. In addition, Chandra *et al.* (2007) and Orabi *et al.* (2013) reported that application of salicylic acid increased soluble protein of cowpea and faba bean plants.

3.3. Effect on oxidative damage and scavenging

3.3.1. Superoxide anion radical (O^-_2) and Electrolyte Leakage (EL)

The stimulation effect of chilling on the value of O^-_2 and E.L % might be attributed to injury of plasma membrane. That damage caused by ROS which could induce Lipid per oxidation and consequently Electrolyte leakage (Hussein and Orabi., 2008 and Kassab *et al*., 2012). Reduction of O^-_2 levels and E.L% in response to ASA treatments as one of the phyto hormones might be (Table 2) due to induction of antioxidant responses that protect the plants from oxidative damage, increased membrane stability and tolerance of plants which in turn enhanced scavenging of harmful free radicals (Sharhrtash *et al*., 2011 and Karlidage *et al*., 2009)

Superoxide (O^-_2) is the primary oxidant, of RNOS since all other RNOS are ultimately derived from its dis mutation or interactions with other reactive species, which themselves go on to induce vascular dysfunction and tissue injury (Kalogres *et al*., 2012). Superoxide (O^-_2) promotes hydroxyl-radical formation and consequent DNA damage in cell of all types. It has been implicated in the production of oxidative DNA damage and lipid peroxidation (Keyer and Imlay., 1996). The content of MDA, a product of lipid peroxidation, has been considered as an indicator of oxidative damage, leading to electrolyte leakage that represents cell membrane injury. Florida cultivar was characterized by significant decreases in the O^-_2 and EL than strain B cultivar in leaf tissues under all treatments (Table 2). Environmental stresses always result in cellular membrane injures including the increase of membrane permeability and MDA content due to oxidative damage (ROS over production) and they are considered to be sensitive stress markers (Orabi, 2004; He *et al*., 2009; Orabi and Mekki., 2008 and Moskova *et al*., 2009).

The decrease of ROS (eg.O^-_2) and electrolyte leakage in tomato leaf tissues under application of ASA is consistent with that reported by Stevens *et al*.(2006) and Agamy *et al*. (2013) who mentioned that SA application regulates and maintains the membrane functions of tomato plants. In addition, SA can diminish the injuries in cell membranes through enhancing the antioxidant potential of plant under stress conditions and partly maintained membrane permeability as well as reduced the amount of ion leakage (Tasgin *et al*., 2006; Orabi *et al*., 2013). Kabiri *et al*. (2014) mentioned that pretreatment with SA was evidenced by a reduction in the level of lipid peroxidation and leakage of electrolytes from plant tissues as well as by more intensive growth processes as compared to control plants.

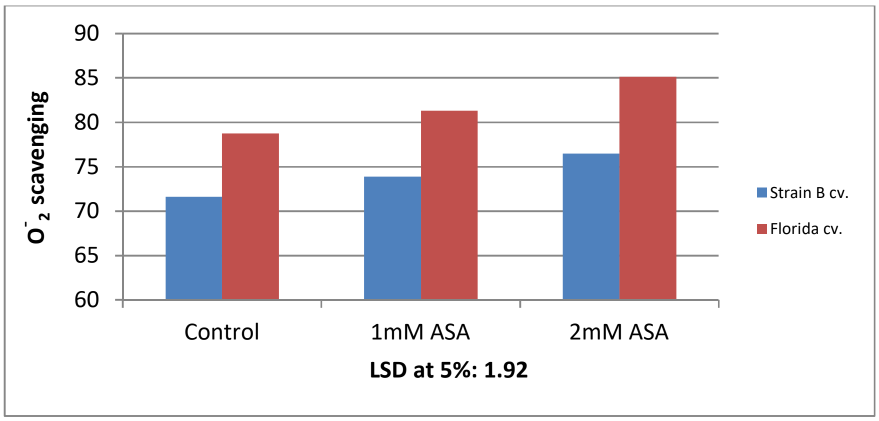
Table 2: Effect of aspirin on superoxide (O^-_2) and Electrolyte Leakage (EL) of two tomato cultivars grown under low temperature conditions

Treatments	O^-2 (A680/g FW)		EL (%)	
	Strain B cv.	**Florida cv.**	**Strain B cv.**	**Florida cv.**
Control	0.91	0.78	59.20	55.27
ASA (1mM)	0.75	0.61	55.41	50.96
ASA (2mM)	0.58	0.42	51.65	45.47
L.S.D 5%	**0.15**		**4.15**	

3.3.2. Superoxide anion radical (O^-_2) scavenging activity

The exposure of tomato plants to chilling stress and treated with ASA (Fig 2) resulted in an obvious increase in Superoxide (O^-2) scavenging activity, in this respect, Ibrahim *et al*, 2013. found that exposure of wheat leaves to UV-B irradiation or heat stress resulted in a more pronounced increase in superoxide anion radical scavenging in tolerant cv *Tritichum aestivum* than in the susceptible cv *Triticum durum* and prolonged exposure of the wheat plants to heat or UV-B stress resulted in a significant reduction in the rate of subsequent superoxide anion radical scavenging activity. Moreover

, Orabi and El Neomani, (2015) demonstrated that either of superoxide onion radical or hydrogen peroxide scavenging were generated in faba bean plants grown under drought stress and further enhanced in response to exogenous proline addition as one of the plant protectant capable to overcome reactive oxygen species (ROS) resulted in plant tolerance. However, the enhancement of Superoxide (O^-2) scavenging activitiey in response to low temperature and further enhanced in response to ASA at minor concertation (Fig 2) revealed and ascertained the occurrence of an oxidative stress that catalyzes the production of reactive oxygen species, consequently accumulating and resulting in several damages which are judged by the criteria and behaviors of the different antioxidants or by other meaning through enzymatic and non enzymatic antioxidants beside protectants(Orabi and El Neomani, 2015). The overproduction of ROS resulted by oxidative stress would be engaged different pathways of the antioxidant system for their removal (Orabi and Abou-Hussein., 2019[a,b] ; Kassab *et al*., 2012; and Orabi *et al*., 2020) where better protection by ASA as a signal agent led to low level of plant damages in this investigation.



AA (Positive control)= 90.96%

Fig 2: Effect of aspirin on Superoxide anion radical (O^-_2) scavenging activity at (150 µg/m) of two tomato cultivars grown under low temperature conditions

3.4. Effect on fruits yield and total soluble solids (TSS):

Florida cultivar was characterized by almostly significant increases in fruits yield (g/plant) than Strain B cultivar under all treatments (Table 3). ASA treatment at 2mM caused the highest significant increase in the yield and TSS of both tomato cultivars followed by ASA treatment at 1mM. The effect of ASA on yield parameters has been reported by with Canakç and Munzuroðlu (2000) who mentioned that acetyl salicylic acid (ASA) administration to the leaf caused an increase in fresh and dry weight gain of radish (*Raphanus sativus* L). These results were in agreement also with those of Singh and Kaur, (1980) on mung bean, Lang, (1986) on *Phaseolus vulgaris* and Singh, *et al.,* 2002 on onion plants. Similar findings were obtained by Resmi and Gopalakrishnan (2004) on cowpea, El-Shrai and Hegazy (2009) on Pea and Abd Ehhamid *et al*. (2016) on wheat and Hussein *et al*. (2015) on onion. These increases in yield due to Aspirin (ASA) effect might be a reflection of the increased growth and development followed by stimulation of some physiological processes and translocation of the product of photosynthesis from source to sink.

Foliar application of SA significantly increased yield and its components of maize (Abd El-Wahed *et al*., 2006), wheat plants (Iqbal and Ashraf, 2006) and Cucumber and tomato fruit yield (Larque-Saavedra; Martin-Mex, 2007 and Orabi *et al*., 2010, 2015)

Fruits of florida cultivar were characterized by significant increases in the TSS than fruits of strain B cultivar (Table 3). ASA treatments (2mM and 1mM) caused significant increases in TSS in fruits of both tomato cultivars relative to control plants. TSS values associated with taste and had significant indication for improvement in yield quality as reported by Vural *et al*. (2000). Chandra *et al*. (2007) and Orabi *et al.* (2018) reported that application of salicylic acid or aspirin increased total soluble sugar of cowpea plants or TSS in mandarin fruits. Moreover, Abdullahi *et al.* (2011) show that plant growth and TSS levels were increased as a result of salicylic acid treatment.

Table 3: Effect of aspirin on fruit yield and total soluble solids of two tomato cultivars grown under low temperature conditions

Treatments	Fruit yield g/plant		Total soluble solids%	
	Strain B cv.	Florida cv.	Strain B cv.	Florida cv.
Control	380.12	419.20	4.34	4.58
ASA (1mM)	541.33	573.28	4.68	5.10
ASA (2mM)	609.40	680.13	5.10	5.75
L.S.D 5%	**55.76**		**0.41**	

El-shraiy and Hegazy (2009) and Raskin *et al*. (1995) reported that ASA treatment could be recommended to induce plant resistance against biotic and abiotic stresses.
Ahmad *et al*. (2012) and Orabi *et al.* (2015) stated that at suboptimal condition of low temperature, priming maize seeds with SA or foliar spray on tomato plants induced activities of scavenging enzymes for increasing the chilling tolerance.

In this concern, ASA can diminish the injuries in cell membranes through enhancing the antioxidant potential of plant under stress conditions and partly maintained membrane permeability as well as reduced the amount of ion leakage. Our results are in a good accordance with Orabi *et al.* (2010) on cucumber Plants, Orabi *et al*., (2015) on tomato plants, Abd Elhamid *et al*. (2016) on wheat plants and Orabi *et al*. (2018) on mandarin fruits. On the other hand, these decrements of reactive oxygen species (Superoxide O^-_2) and the ability of its perfect scaveniging activity have occurred as a result of ASA application ascertain that plant tolerance would be attained to scavenge ROS produced under low temperature.

4. Conclusion

It could be concluded that ASA (1, 2 mM) treatments have positive effect on growth, protectant concentrations, antioxidant enzymes (APX, GR and Gly1) activity and specific activity and fruits yield of tomato plants grown under low temperature conditions. On the other hand, these treatments caused obvious decreases in O_2^- and EL values. It is worthy to mention that, ASA treatment at 2.0 mM was the most pronounced treatment.

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