Ann. Bangladesh Agric. (2021) 25 (1) : 55-65 *ISSN 1025-482X (Print)*
www.doi.org/10.3329/aba.v25i1.58155 *2521-5477 (Online)*

ASCORBIC ACID INFLUENCES ON GROWTH AND YIELD OF TOMATO

M. A. Rahman[1], M. Zakaria[1], M. A. B. Mia[2] and M. S. Biswas[1*]

Abstract

Reactive oxygen species (ROS) generated in various metabolic reactions in plants are mostly known for toxicity. Whereas ascorbic acid (AsA) involves in various physiological and biochemical processes; and acts as a ROS scavenger. Therefore, the experiment was conducted to investigate the effects of exogenous application of AsA on growth and yield of tomato plants. The ROS scavenger AsA was applied in the leaves of tomato plants in four treatment combinations (T_1-Control; T_2-0.5 mM; T_3-2.0 mM and T_4-4.0 mM) with three replications. Among the treatments, AsA at 4.0 mM (T_4) efficiently increased leaf length (12%), inflorescence length (17%), and flower number (29%) than the untreated control plants. AsA at 0.5 and 2.0 mM also showed positive effects on the plant morphology to a lesser extent. Importantly, AsA at 4.0 mM increased yield by 18% than the control treatment. In physiochemical parameters, AsA at 4.0 mM (T_4) was retained 64.07% higher chlorophyll content than the control plant even after 60 days of application. Exogenous application of AsA effectively reduced oxidative stress of the plant, which was attributed to the less accumulation of H_2O_2 and lipid peroxidation of membrane lipids. AsA at 4.0 mM decreased H_2O_2 and MDA accumulation by 39% and 45%, respectively, compared with the control plants. Therefore, exogenous application of AsA is a useful tool to enhance growth and yield of tomato under field conditions by lowering the accumulation of ROS and lipid peroxidation.

Keywords: Lipid peroxidation, oxidative stress, ascorbate, antioxidants, *Solanum lycopersicum.*

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most popular edible and nutritious vegetable for the family Solanaceae. The nutritive value of the fruit is an important aspect of the quality of tomatoes. Tomato is universally treated as protective food since it is rich in vitamins, minerals, and antioxidant properties (Sekhar *et al.,* 2010). Tomato is cultivated in all parts of Bangladesh as its adaptability to a wide range of soil and climate (Haque *et al.,* 1999). It is cultivated as a winter vegetable with an annual production of about 3.9 lac metric tons (BBS, 2018). Tomato is also cultivated in summer in recent times due to the development of widely adapted varieties in a range of hot and wet conditions in Bangladesh (Ahmad, 2002). However, the plants are grown in the winter and summer seasons are exposed to various environmental changes that affect plant growth and development. Therefore, providing a suitable

[1]Department of Horticulture, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706, Bangladesh. [2]Department of Crop Botany, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706, Bangladesh. *Corresponding author: sanaullah@bsmrau.edu.bd

environment is a prerequisite to harvest maximum yield.

As sessile organism, plants are vulnerable in the changing environments. To adapt in the challenged environments, plants evolved extensive mechanism for adaptation. In response to various environmental stimuli plant cell accumulated reactive oxygen species (ROS) contribute to major physiological processes in plants (Waszczak *et al*., 2018). ROS such as superoxide radical (O_2^{-1}), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$) are inevitably generated in plant cells as a consequence of normal metabolism. Higher accumulation of ROS acts as an initiator of cell death (Mittler *et al.,* 2011). ROS involvement is also relevant to certain developmental types of cell death, such as the development of tracheary elements in the xylem of vascular plants (Kuriyama and Fukuda, 2002), leaf senescence (Zapta *et al*., 2005). ROS are highly reactive molecules capable of causing oxidative damage to protein, DNA, and lipids (Biswas and Mano, 2015, 2016; Mano *et al*., 2019). As well as being toxic molecules, ROS also act as important signaling molecules regulating many important biological processes, such as growth, development, and responses to abiotic and biotic stresses (Mano *et al.,* 2019). Thus, it is important to maintain the balance between the generation and scavenging of ROS.

Plant cells are equipped with abundant antioxidant molecules to avoid the damaging effects of ROS. The reduced form of glutathione (GSH), ascorbic acid (AsA), AsA and phenolic substances provide the first defense from oxidative stress. Thus, the balance between ROS production and the antioxidants protection system is critically important for various plant physiological processes (Mano *et al.,* 2019; Gill and Tuteja, 2010). Despite an efficient antioxidant system, oxidative damages still occur in plant cells either due to runaway production or inefficient scavenging of ROS. The senescence of green plant tissues is generally accompanied by ROS production and increased contents of lipid peroxides (Wojciechowska *et al.,* 2018). The unsaturated bonds of the fatty acid moiety of lipids are readily oxygenated to form lipid peroxides (LOOHs). LOOHs are degraded enzymatically or non-enzymatically to form various aldehydes and ketones (Farmer and Mueller, 2013). Thus, the accumulation of ROS and their downstream products, aldehydes and ketones, accelerate plant senescence.

Hence, this study aims to assess the effectiveness of various concentrations of AsA that lower the accumulation of ROS, and subsequently decrease lipid peroxidation in plants and ripening fruits.

Materials and Methods

The experiment was carried out at the Department of Horticulture of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur 1706, Bangladesh. The soil of the experimental site was sandy loam in texture and acidic (pH 5.8). The land was well prepared with the tractor, followed by laddering until good tilth. The experiment was laid out in the Randomized Complete Block Design (RCBD) with three replications. The unit plot size was 3.0 m x 1.0 m following 60 cm x 50 cm plant spacing. The variety BARI Tomato 14 was used in this study. In each plot having 6 plants and total

72 plants were accommodated. The tomato seedlings were germinated in a plastic tray for 10 days. Then the seedlings were transferred to polybag for another 20 days. Healthy and uniform seedlings of 30 days were transplanted in the fields.

ROS scavenger AsA was applied in four treatment combinations viz. Control (water, T_1), 0.5 mM AsA (T_2), 2.0 mM AsA (T_3) and 4.0 mM AsA (T_4). Freshly prepared AsA at the doses mentioned above were spraying on the 21-, 35-, and 49-days of transplanting tomato seedlings in the fields using a hand sprayer. In the control plot, only distilled water was applied instead of AsA. Fertilizer doses and various intercultural operations such as gap filling, weeding, staking and pruning, irrigation, remove excess branches, insect and pest management were accomplished for better growth and development of the plants following Hand Book of Agricultural Technology (Chowdhury and Hassan, 2013). Fruits were harvested at four day-interval during the early ripening stage when they attained red colour and calculated cumulative weight to estimate the yield of tomato.

Collection of field data

Various morphological data were collected from five randomly selected plants of each replication of treatments. Leaf length and inflorescence length was measured with a measuring scale, and the average was calculated. Flower cluster per plant and fruits per plant of each replication was counted, and the average was calculated. Fruit yield per plant from each replication was recorded, and the average yield of five plants were calculated in each plot.

Collection of physiochemical data

Total chlorophyll content

A portable chlorophyll meter (SPAD-502, Minolta Corporation, Ltd., Osaka, Japan) was used to measure the leaf chlorophyll content. The chlorophyll content was recorded at the middle of leaflets of the third leaf from the top. Twelve random measurements per plot were taken and averaged to a single SPAD value for each treatment. The measured chlorophyll content was expressed as a percentage (%) (Yuan *et al*., 2016). It was estimated three times and made an average as the final data.

Measurement of hydrogen peroxide (H_2O_2) and lipid peroxidation (malondialdehyde-MDA) levels

Hydrogen peroxide (H_2O_2) concentration was determined according to Loreto and Velikova (2001). Leaf samples of 0.2 g were homogenized in 3 ml of 1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000 rpm for 10 min at 4°C. Subsequently, 0.75 ml of the supernatant was added to 0.75 ml of 10 mM phosphate buffer (pH 7.0) and 1.5 ml of 1 M KI. H_2O_2 concentration was evaluated by comparing its absorbance at 390 nm to a standard calibration curve. The concentration of H_2O_2 was calculated from a standard curve plotted in the range from 10 to 100 µmol ml^{-1}. H_2O_2 concentration was expressed as µmol g^{-1} FW. MDA content was determined by the thiobarbituric acid (TBA) reaction as described by Ali *et al.* (2005), with slight modifications. Fresh leaf samples (0.2) were ground in 5 ml of 0.1% trichloroacetic acid (TCA) and centrifuged at 14,000 rpm for 15 min. After centrifugation, 1 ml of the supernatant was mixed with 2.5 ml 0.5% TBA in 20% TCA

and incubated in hot water (95°C) for 30 min. After that, it was cooled immediately on ice to stop the reaction and centrifuged at 10,000 rpm for 30 min. Absorbance at 532 and 600 nm was determined, and MDA concentration was estimated by subtracting the non-specific absorption at 600 nm from the absorption at 532 nm, using an absorbance coefficient of extinction (155 mM^{-1} cm^{-1}).

Data analysis

The observations for leaf and inflorescence length and yield contributing characters such as flower cluster and number of fruits per inflorescence were statistically analyzed to determine the significance of variation resulting from the experimental treatments. The collected data were analyzed by using Analysis of Variance, and treatment means were compared using Least Significant Difference (LSD) in the Statistix 10 program.

Results and Discussion

Effect of exogenous AsA on the leaf and inflorescence length of tomato plant

In the present study, the effects of foliar spray of AsA on the leaf and inflorescence length of the tomato plant was measured. The result showed that all the AsA treated plants produced longer leaf than the untreated plants. However, AsA at 4.0 mM produced the longest leaf (39.49 cm) at 60 days of spraying. AsA at a lower dose (2.0 mM) also produced a significantly larger leaf (33.45 cm) at 45 days of transplanting (Table 1). In addition to the plant growth regulators, different antioxidants molecules such as AsA are also recently using to enhance plant growth. Foliar spray of AsA increased plant height and biomass accumulation, 4.2 % and 11%, even under stressful conditions in rice plants (Hassan *et al.,* 2021).

Inflorescence length is an important attribute that may affects yield. At 45 days of transplanting, the ranges of inflorescence length varied from 9.66 cm to 11.44 cm but were statistically identical although 4.0 mM (T_4) produced the longest one (11.00 cm), and the shortest one (11.44 cm) was found in the control plant (T_1). At 60 days of application, AsA at 0.5 mM, 2.0 mM and 4.0 mM also produced the identical inflorescence length (13.66 cm), and the control plant produced the shortest one (11.67 cm) (Fig. 1). Briefly, the exogenous application of AsA at all doses (0.5, 2.0, and 4.0 mM) were significantly increased inflorescence length by about 18% and 17% after 45 and 90 days of spraying (Fig. 1).

Table 1. Effect of AsA on the leaf length of tomato at different days after transplanting

Treatments	Leaf length (cm) at different days after transplanting (DAT)		
	30 DAT	45 DAT	60 DAT
Control, 0 mM AsA	13.56^{a}	28.44^{c}	35.44^{b}
0.5 mM AsA	13.33^{a}	30.22^{bc}	37.89^{b}
2.0 mM AsA	13.44^{a}	31.89^{ab}	35.78^{b}
4.0 mM AsA	13.67^{a}	33.11^{a}	39.89^{a}
$LSD_{0.05}$	1.01	1.86	3.47

Means bearing the same letter(s) in a column do not differ significantly at a 5% probability level.

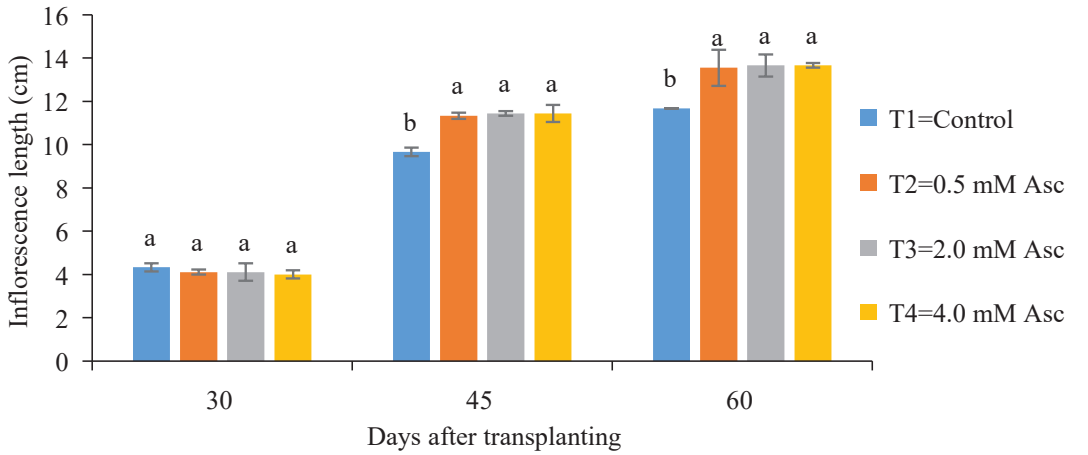

Fig. 1. Effect of different doses of AsA on the inflorescence length of tomato. The full-grown inflorescence just started to fruit setting were selected for the measurement of inflorescence length. Bars represent the means ± standard errors of three independent experiments. Differences among treatments were analyzed by LSD. P<0.05.

The effect of AsA was not found at 30 days of application. But the growth-promoting effects of AsA were observed in all the tested doses and after 30 days of application. Several recent reports showed that foliar application of AsA increased yield and yield components in many plant varieties. AsA at 500 mg L^{-1} was applied twice at 30- and 60-days intervals and found increased plant growth and grain yield in wheat (Mohamed, 2013).

Effect of different levels of AsA on the number of the flower cluster and fruits per plant

It is evident from the data (Table 2) that AsA at all of the doses (0.5, 2.0, and 4.0 mM) significantly influenced the number of flower clusters at 30, 45, and 60 days after transplanting. The maximum number of flower clusters were observed at 30, 45, and 60 days after transplanting. AsA at 4.0 mM (T_4) effectively produced an increased number of flower clusters in the inflorescence by 30, 32.36 and 29.28% compared with the control plants (T1) at 30, 45 and 60 days after transplanting. Thus, AsA at 4.0 mM was an effective dose to increase flower cluster per inflorescence of the tomato plant. The effect of foliar spay of AsA at 50 and 100 mg L^{-1} in *Hibiscus rosasinesis* L. was observed, and they reported that AsA increased the number of flowers per plant (Fatma *et al.,* 2009).

Data on the number of fruits per plant were recorded on 30, 45, and 60 days after transplanting (Fig. 2). A significant variation was observed in the number of fruits per plant among the treatments. AsA at 4.0 mM produced the highest number of fruits per plant at 30, 45, and 60 days after transplanting. Spraying of AsA by 0.5 and 2.0 mM were also increased fruits number but were identical at 30 and 45 days of transplanting. However, AsA (0.5, 2.0, and 4.0 mM) increased the number of fruits at 60 days of transplanting. Exogenous effects

Table 2. Effect of AsA on the number of flower cluster per plant at different days after transplanting of tomato

Treatments	Flower cluster per plant at different days after transplanting		
	30 DAT	45 DAT	60 DAT
Control, 0 mM AsA	5.44[b]	8.22[b]	13.66[b]
0.5 mM AsA	6.33[b]	10.67[a]	15.33[b]
2.0 mM AsA	7.11[ab]	10.11[a]	14.89[b]
4.0 mM AsA	7.22[a]	10.88[a]	17.66[a]
$LSD_{0.05}$	1.55	1.62	2.68

Means bearing the same letter(s) in a column do not differ significantly at a 5% probability level.

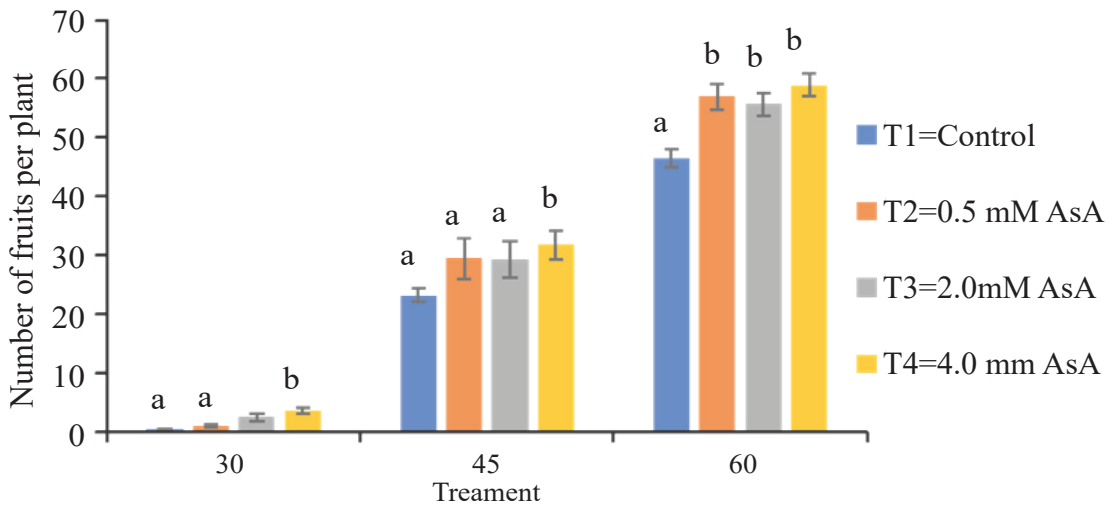

Fig. 2. Effect of different doses of AsA on the number of fruits per plant of tomato. The total number of fruits was counted at the indicated days of observation. Means ± SE of three independent experiments is shown. Differences among treatments were analyzed by LSD. P<0.05.

of AsA on the flower and fruit number were not studied in tomatoes. However, AsA at various doses (100, 200 and 300 mg L^{-1}) were applied in wheat (*T. aestivum* L.) and found an increased number of tillers, spikes per plant, spikelets/spike, and grain yield (Bakry *et al.,* 2013; Mohamed, 2013).

Fruit yield per plot

Fruit yield per plot was recorded cumulatively throughout the growing season. The application of AsA at different doses significantly affect fruit yield (Fig. 3). Fruit yield of different treatments ranged from 21.90 kg to 25.83 kg per plot. The maximum fruit yield (25.83 kg) per plot was recorded in T_4 (4.0 mM AsA) treatment, which was identical with T3 (2.0 mM AsA, 22.14 kg) and the lowest fruit weight (21.90 kg) was obtained from the T1 (control) (Fig. 3). Exogenous application of AsA at various growth stages

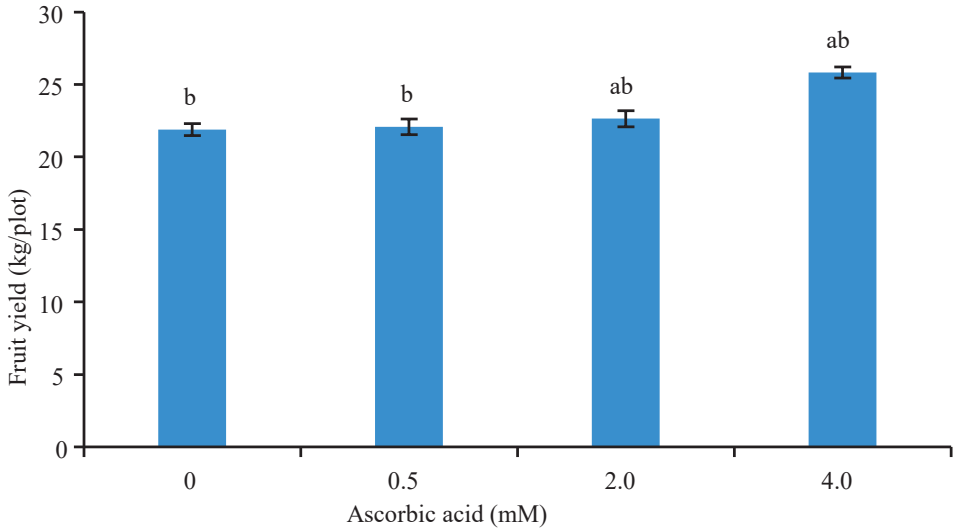

Fig. 3. Effect of different doses of AsA on the yield of tomato per plot (2.40 m^2). Means ± SE of three independent experiments is shown. Means bearing the same letter(s) do not differ significantly at 5% level of probability. Differences among treatments were analyzed by LSD.

in different plant varieties improves yield by regulating physiochemical properties of plant (Mukhtar *et al.,* 2016; Akram *et al.,* 2017). Foliar application of AsA at 100, 200, and 300 mg L^{-1} doses were increased plant height, seed yield in chickpea (*Cicer arietinum* L.) (Zarghamnejad *et al.,* 2014).

Chlorophyll content

The total chlorophyll content measured by SPAD meter showed that untreated control plants were inefficient to prevent chlorophyll degradation. However, the plants received exogenous addition of AsA protected chlorophyll degradation throughout the growing season. It revealed that AsA at all doses (0.5, 2.0 and 4.0 mM) increased chlorophyll contents by about 6% and 8% at 30 and 60 days of transplanting (Fig. 3). After 45 days of transplanting AsA also showed an increasing trend of chlorophyll protection compared with the control plants (Fig. 4). The maximum chlorophyll content (64.87%) was recorded at AsA at 2.0 mM (T3) treated plants after 60 days of transplanting, while the minimum chlorophyll content (55.6%) was recorded from the control plants. Thus AsA at 2.0 and 4.0 mM were effective in retaining the chlorophyll content of tomato plants. It was reported that foliar spray of AsA at 0.5 mM in the seedling stage of tomato significantly increased the total chlorophyll content compared with the untreated and heat stressed tomato plants (Alyafi, 2019). Exogenous application of AsA at 100 mM also showed improved chlorophyll content in common bean (*Phaseolus vulgaris* L.) (Dolatabadian *et al.,* 2009).

Endogenous H_2O_2 and MDA levels in the leaves

It revealed from the above results and discussion that exogenous application of AsA promoted plant growth and increased

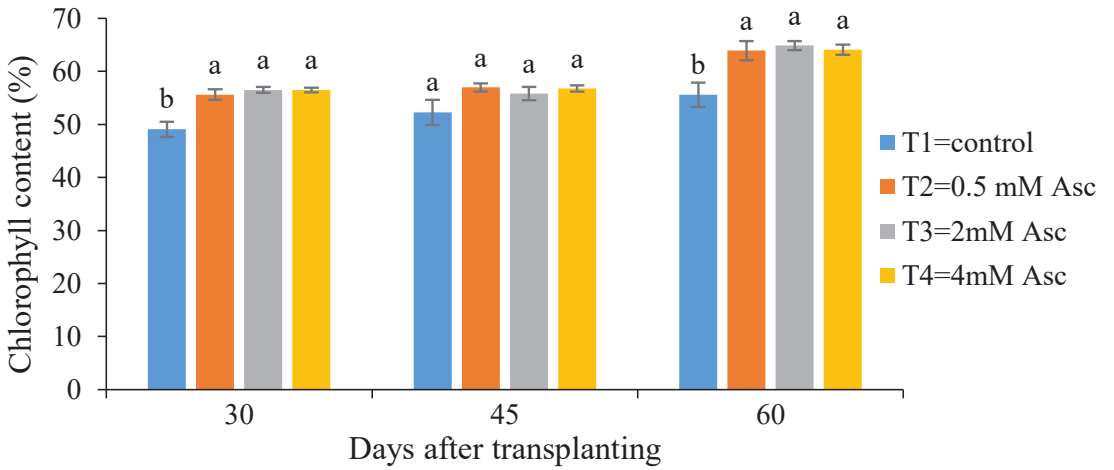

Fig. 4. Different doses of AsA influence the chlorophyll content of tomato leaves at different days after transplanting. The full-grown leaves were selected for the determination of chlorophyll content by the SPAD meter. Means ± SE of three independent experiments is shown. Differences among treatments were analyzed by LSD. P<0.05.

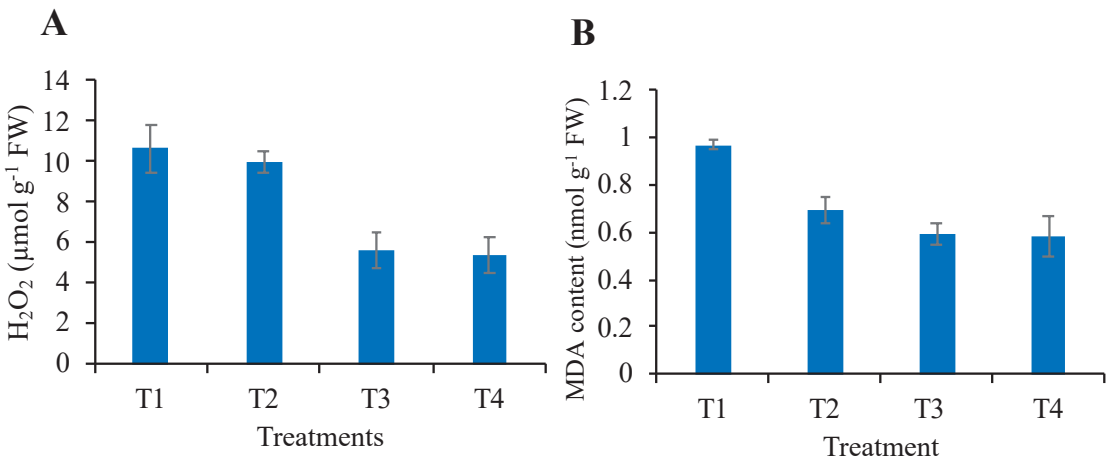

Fig. 5. Effect of different doses of AsA on the endogenous level of (A) H_2O_2 and (B) MDA in tomato leaves. The full-grown leaves were selected for H_2O_2 and MDA quantification after 45 days of AsA spraying at different doses; T1 = Control, T2 = 0.5 mM, T3 = 2.0 mM, and T4 = 4.0 mM. Means ± SE of three independent experiments is shown. Differences among treatments were analyzed by LSD. P<0.05.

yield of tomato. Therefore, it is assumed that AsA could alleviate oxidative stress, which affects plant performance. It was found that the highest level of H_2O_2 was accumulated in the untreated control plants (10.67 µmol g^{-1} FW), and the lowest accumulation (5.33 µmol g^{-1} FW) was found in the AsA at 4 mM (T4) treated plants. AsA at 2.0 mM also lowered

endogenous H_2O_2 contents (Fig. 5A). A similar accumulation tendency of lipid peroxidation was also observed in MDA levels. Oxidative damage of leaf lipids by the accumulation of H_2O_2 resulted in MDA. The highest MDA (0.871 nmol g^1 FW) was recorded in the control treatment (T1) and the lowest MDA (0.4537 nmol g^1 FW) was recorded in T4 treatment (Fig. 5B). These observations suggest that the exogenous application of AsA is efficient in reducing oxidative stress, which influences plant growth and yield performance. Exogenous application of AsA improved morpho-physiological characteristics, ions accumulation in the roots and shoots, and increased antioxidant capacities to alleviate oxidative stress in barley (Hassan *et al.,* 2021). The addition of AsA has significantly increased antioxidant properties and decreased accumulation of H_2O_2 and MDA content in plant tissues, thus improving plant abiotic stress tolerance (Zhou *et al.,* 2016). Thus, enhancing the antioxidants system to maintain a lower level of ROS and MDA are critically important for plant growth and development.

Conclusion

Reactive oxygen species (ROS) generated in various metabolic processes in plants are important for its reactivity with cellular molecules. Besides its toxicity with cellular molecules ROS are also involved in different physiological processes in plants. In order to observe the effects of ROS scavenger in the growth and yield of tomato, AsA at four doses with three installments were applied in the field-grown plants. The exogenous application of AsA at 4.0 mM (T4) significantly increased the leaf length, inflorescence length and flower number of the plants, which eventually increased the yield of tomato by 18% compared with the untreated. The influence of AsA in the endogenous biochemical changes have also been verified. It showed that AsA at 4.0 mM greatly increased the chlorophyll content by 57.46% and 64.07% after 30 and 60 days of transplant compared with the untreated plants. Importantly, AsA at 4.0 mM (T4) was reduced H_2O_2 by 39% and MDA by 45% than the untreated plants. AsA-induced reduction of H_2O_2 and MDA accumulation and retention of chlorophyll contents may attribute to increased leaf length, inflorescence length, flower number, and yield of the plants. Therefore, exogenous application of AsA is an efficient management practices to maintain oxidative balance of the plant cells.

Acknowledgments

This research was funded by the Research Management Wing (RWM) of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706, Bangladesh.

References

Ahmad, S. 2002. Genetics of fruit set and related traits in tomato under hot-humid conditions (Ph. D. Thesis). Pp. 44-180. Bangabandhu Sheikh Mujibur Rahman Agricultural University. Salna, Gazipur.

Akram, N. A., F. Shafiq and M. Ashraf. 2017. Ascorbic acid-A potential oxidant scavenger and its role in plant development and abiotic stress tolerance. *Front. Plant Sci.* 8: 613.

Alayafi, A. A. M. 2020. Exogenous ascorbic acid induces systemic heat stress tolerance in tomato seedlings: Transcriptional regulation mechanism. *Environ. Sci. Pollut. Res.* 27(16): 19186-19199.

Ali, M. B., E. J. Hahn and K. Y. Paek. 2005. Effects of temperature on oxidative stress defense systems, lipid peroxidation and lipoxygenase activity in Phalaenopsis. *Plant Physiol. Biochem.* 43(3): 213-223.

Bakry, A. B., R. E. Abdelraouf and M. A. Ahmed. 2013. Effect of drought stress and ascorbic acid foliar application on productivity and irrigation water use efficiency of wheat under newly reclaimed sandy soil. *Agriculture.* 57: 14398–14403.

BBS. 2018. Yearbook of Agricultural Statistics of Bangladesh. Ministry of Planning, Government of the People's Republic of Bangladesh 151 P.

Biswas, M. S., and J. Mano. 2015. Lipid peroxide-derived short-chain carbonyls mediate hydrogen peroxide-induced and salt-induced programmed cell death in plants. *Plant Physiol.* 168: 885–898.

Biswas, M. S., and J. Mano. 2016. Reactive carbonyl species activate caspase-3-like protease to initiate programmed cell death in plants. *Plant Cell Physiol.* 57: 1432–1442.

Chowdhury, M. A. H. and M. S. Hassan. 2013. Hand Book of Agricultural Technology. Bangladesh Agricultural Research Council, Farmgate, Dhaka 230 P.

Dolatabadian, A., S. A. M. Modarres Sanavy and M. Sharifi. 2009. Alleviation of water deficit stress effects by foliar application of ascorbic acid on *Zea mays* L. *J. Agron. Crop Sci*. 195(5): 347-355.

Farmer, E. E. and M. J. Mueller. 2013. ROS-mediated lipid peroxidation and RES-activated signaling. *Annu. Rev. Plant Biol.*. 64: 429–450.

Fatma, E., M. El-Quesni, A. El-Aziz, G. Nahed and M. M. Kandil. 2009. Some studies on the effect of ascorbic acid and a-tocopherol on the growth and some chemical composition of *Hibiscus rosasineses* L. at Nubaria. *Ozean J. Appl. Sci.* 2: 159–167.

Gill, S. S. and N. Tuteja. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem. 48*(12): 909-930.

Haque, M. A., A. K. M. A. Hossain and K. U. Ahmed. 1999. A comparative study on the performance of different varieties of tomato. II. Varietal response of different seasons and temperature in respect of yield and yield components. *Bangladesh Hort. 26:* 39-45.

Hassan, A., S. F. Amjad, M. H. Saleem, H. Yasmin, M. Imran and M. Riaz. 2021. Foliar application of ascorbic acid enhances salinity stress tolerance in barley (*Hordeum vulgare* L.) through modulation of morpho-physio-biochemical attributes, ions uptake, osmo-protectants and stress response genes expression. *Saudi J. Biol. Sci.* 28(8): 4276-4290. doi: 10.1016/j.sjbs.2021.03.045.

Kuriyama, H. and H. Fukuda. 2002. Developmental programmed cell death in plants. *Curr. Opin. Plant Biol.* 5: 568–573.

Loreto, F. and V. Velikova. 2001. Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. *Plant Physiol.* 127: 1781-1787.

Mano, J., M. S. Biswas and K. Sugimoto. 2019. Reactive carbonyl species: a missing link in ROS signaling. *MDPI Plants*. 8: 391.

Mittler, R., S. Vanderauwera, N. Suzuki, G. A. D. Miller, V. B.Tognetti, K. Vandepoele, M. Gollery, V. Shulaev and F. V. Breusegem. 2011. ROS signaling: the new wave? *Trends Plant Sci.* 16(6): 300-309.

Mohamed, N. E. M. 2013. Behaviour of wheat cv. Masr-1 plants to foliar application of some vitamins. *Nat. Sci.* 11: 1–5.

Mukhtar, A., N. A. Akram, R. Aisha, S. Shafiq and M. Ashraf. 2016. Foliar applied ascorbic acid enhances antioxidative potential and

drought tolerance in cauliflower (*Brassica oleracea* L. var. Botrytis). *Agrochimi*. 60: 100-113.

Sekhar, L., B. G. Prakash, P. M. Salimath, C. P. Hiremath, O. Sridevi and A. A. Patil. 2010. Implications of heterosis and combining ability among productive single cross hybrids in tomato. *Electron. J. Plant Breed.* 1(4): 706-711.

Yuan, Z., Q. Cao, K. Zhang, S. T. Ata-Ul-Karim, Y. Tian, Y. Zhu, W. Cao and X. Liu. 2016. Optimal leaf positions for SPAD meter measurement in rice. *Front. Plant Sci.* 7:719.

Waszczak, C., M. Carmody, and J. Kangasjärvi. 2018. Reactive oxygen species in plant signaling. *Annu. Rev. Plant Biol.* 69: 209-236.

Wojciechowska, N., E. Sobieszczuk-Nowicka and A. Bagniewska-Zadworna. 2018. Plant organ senescence – regulation by manifold pathways. *Plant Biol.* 20(2): 167-181.

Zapata, J. M., A. Guéra, A. Esteban-Carrasco, M. Martín and B. Sabater. 2005. Chloroplasts regulate leaf senescence: Delayed senescence in transgenic ndhF-defective tobacco. *Cell Death Differ.* 12: 1277-1284.

Zarghamnejad, S., A. Rokhzadi, and K. Mohammadi. 2014. Chickpea response to ascorbic acid foliar application at vegetative and reproductive stages. *Int. J. Biosci.* 5: 166-170.

Zhou, X., Z. Gu, H. Xu, L. Chen, G. Tao, Y. Yu, and K. Li. 2016. The effects of exogenous ascorbic acid on the mechanism of physiological and biochemical responses to nitrate uptake in two rice cultivars (*Oryza sativa* L.) under aluminum stress. *J. Plant Growth Regul*. 35(4): 1013-1024.