Research Article

The effect of ascorbic acid on rooting of *Tradescantia fluminensis* cuttings

Leila Zarandi-Miandoab [1*]

[1] Faculty of basic sciences, Department of Plant Biology, Azarbaijan Shahid Madani University, Tabriz, Iran

*Corresponding author: Leila Zarandi-Miandoab, Ph.D. zarandi@azaruniv.ac.ir

Received 7th September 2021 Revised 19th November 2021 Accepted 25th January 2022

Abstract

Ascorbic Acid (AsA) is a powerful antioxidant, vitamin, and enzyme cofactor that has significant effects on plant growth and development. A study was performed to investigate the effects of AsA on the formation and growth of adventitious roots in cuttings of *Tradescantia fluminensis*. The plants were treated with two levels of AsA (0, and 0.5 mM). Lengths and numbers of adventitious roots, free amino acid content, hydrogen peroxide content, ascorbate-peroxidase activity, and AsA/ Dehydroascorbate (DHA) ratio were measured. Application of 0.5 mM AsA reduced the number of adventitious roots but did not affect the lengths of adventitious roots. Supplemental AsA leads to the reduction of free amino acids and hydrogen peroxide contents, and AsA/DHA ratio in comparison to control plants. The activity of ascorbate-peroxidase was increased under AsA application. The addition of AsA to the rooting medium delayed the formation time of adventitious roots in *T. fluminensis*. The results suggest that the differentiation of parenchymal cells into tracheids during the use of AsA delays because the H_2O_2 and amino acids are required for the lignification of secondary cell walls. We assumed that increasing the activity of APX results in AsA/DHA ratio reduction by the addition of AsA to the rooting medium.

Keywords: Ascorbic Acid (AsA), Dehydroascorbate (DHA), Ascorbate peroxidase (APX)

Introduction

Some species of Tradescantia are cultivated as ornamental plants because of the beautiful color of the leaves. In the genus Tradescantia (Commelinaceae family), the leaves are found in different colors such as plain green, green with streaks of cream, pink, purple, and even completely purple. Small-leaf spiderwort (*Tradescantia fluminensis*) is a perennial sub-succulent herb that creeping stems are used as flooring and especially in hanging baskets. Growing Tradescantia is very easy (Seitz and Clark, 2016) and the most important method for propagation of the plant is cutting. In propagation by cuttings, some parts of the vegetative organs such as stems, roots, or leaves are separated and rooted after being placed in suitable conditions (Peter and Burdick, 2010). Roots created in this way are adventitious and are induced by wounds, for example by separation from the mother plant (Garrod *et al.*, 1982).

The physiological and environmental factors affect the formation of adventitious roots after cutting. Each of these factors may have a positive or negative effect on the rooting of a plant species, so during preparing, planting, and maintaining cuttings, one should be paid enough attention to all the effective factors in rooting. The rooting of cuttings in different plants varies and in a particular plant depending on the type of cuttings, the presence of a certain concentration of plant hormones (especially auxins), the ratio of metabolites, and vitamins (Seitz and Clark, 2016).

AsA or vitamin C is one of the water-soluble vitamins that is found at high concentrations in plant tissues (fresh fruits and vegetables). AsA plays an important role in plants as a powerful and active antioxidant. By modifying gene expression, AsA can play a crucial role in defense functions as well as plant survival (Pastori *et al.*, 2003). New research indicates that AsA is involved in several physiological processes such as plant protection against oxidative stress, key enzyme activities, cell division, development, and aging (Horemans *et al.*, 2000; Horemans *et al.*, 2000; Matic, 2014). However, the multifunctional nature of AsA has made it difficult to accurately describe its role under a specific physiological process .

AsA is involved in the elimination of free radicals during xylogen synthesis, the polymerization of xylogen monomers, and the lignification of the cell walls. Ascorbate peroxidase (APX) maintains cell wall flexibility by removing H_2O_2 (Matic, 2014). AsA reversibly controls the activity of APX in the apoplast, prevents the release of free radicals into the apoplast, and regulates the lignification of plant cell walls (De Cabo *et al.*, 1996). Polymerization of xylogen monomers, and cell wall lignification are regulated by a balance between AsA and H_2O_2 (Davey *et al.*, 2000). Monohydroascorbate, formed by the oxidation of ascorbate, is reduced by cytochrome b on the plasma membrane and promotes cell growth (Smirnoff, 2011). AsA and DHA affect the binding of cell wall proteins and polysaccharides and the cell wall strength (Padh, 1990). Exogenous mono-hydro ascorbate promotes cell growth and rooting of onions. AsA acts directly as an effective electron donor to scavenging ROS (Pignocchi *et al.*, 2003). One of the key roles of AsA in plant cells is to protect chloroplasts from oxidative damage. AsA can remove reactive oxygen species (ROS) that are produced directly or indirectly during various physiological processes such as photosynthesis, oxidation metabolism, and stress response, thus protect plants from damage (Smirnoff and Wheeler, 2000).

The ascorbate-glutathione cycle (Figure 1) is one of the most important antioxidant systems in plants. In this cycle, AsA and glutathione are used as reductants and are recycled through the use of ATP and NAD(P)H (Noctor and Foyer, 1998).

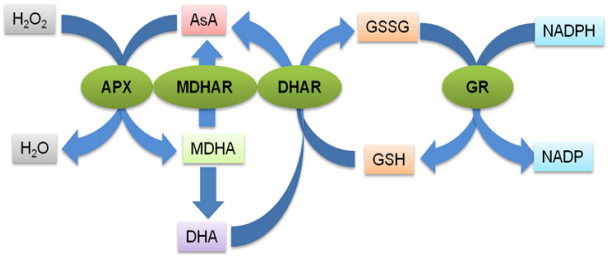

Figure 1. Ascorbate-glutathione cycle

APX is an enzyme involved in the oxidation of AsA and H_2O_2 deletion (which is present in various subcellular parts such as cytoplasm, chloroplast, mitochondria, and peroxisome) (Jimenez *et al.*, 1997) and produces DHA by AsA oxidation. H_2O_2 is produced during photosynthesis, respiration, as well as under various stresses such as drought, extreme temperatures, salt, UV, salinity, and wounds. Excess H_2O_2 causes damage to lipids, nucleic acids, and proteins, and oxidative damage ultimately leads to physiological disorders and premature cell death (Wang *et al.*, 2003). Therefore, in this study, to gain a better insight into adventitious roots formation under exogenous supplemental of 0.5 mM AsA, some biochemical and anatomical parameters in spiderwort (*Tradescantia fluminensis* L.) cuttings were investigated.

Materials and Methods

The plant used in this study can be seen in Figure 2. At first, healthy cuttings of spiderwort were selected, cut, and transferred to hydroponic media-containing solutions with concentrations of zero and 0.5 mM AsA (3 replications). The number of adventitious roots was counted, and their length was measured with a ruler during the first, third, and seventh days, and other analyzes were performed only on the seventh day.

Figure 2- *Tradescantia fluminensis*

Assessment of total free amino acid content: 0.16 g of the fresh root was homogenized in 6 ml of cold potassium phosphate buffer (0.05M and pH=7.5). After centrifugation, 5 ml of the solution was mixed with 1 ml of Ninhydrin (2,2-dihydroxyindane-1,3-dione) reagent and placed in a boiling water bath for 5 minutes. After 30 minutes, the absorbance was determined at 570 nm (Harding and MacLean, 1916). The final value was reported based on mg/g of fresh weight.

Measurement of H_2O_2 content: 0.2 g of the fresh root was homogeneous in 3 ml of potassium phosphate (50 mM and pH=6.5) and centrifuged at 4000 rpm for 25 minutes. 1.5 ml of topical extract was mixed with 1% titanium chloride and centrifuged for another 25 minutes. The adsorption rate of the supernatant was determined at 410 nm and the H_2O_2 content was calculated using a correction factor of 0.28µM cm^{-1}. The final results were reported as μmol/g fresh weight (Chaparzadeh *et al.*, 2004).

Enzyme extraction: 0.2g of fresh root homogenized in 2ml ice-cold potassium phosphate (100 mM, pH=7.5, 1% PVP, 1% EDTA). It was then centrifuged at 13,000 rpm for 15 minutes at 4°C. The supernatant was stored on ice to measure the activity of APX.

Assessment of APX activity: The solutions were prepared as follows: first solution A containing AsA (250 µM), potassium phosphate buffer (pH=7, 50 mM), Na_2EDTA (0.5 mM), and the solution containing H_2O_2 (1.5 mM). And potassium phosphate buffer (pH 7, 50 mM) was prepared. Pour 0.45 ml of solution A and 0.45 ml of solution B into the cuvette and then pour 0.1 ml of the enzyme extract, absorbance at 290 nm was recorded and read again after 60 seconds. The activity of APX was calculated using a quenching coefficient of 2.8 $mM^{-1}cm^{-1}$. Final results were reported as enzymatic units per mg of protein (Qian *et al.*, 2013).

Assessment of ascorbate, total AsA, and DHA content: 0.13 g of root tissue was homogenized with 2 ml of 6% TCA on ice and then extract was placed in ice in a mortar for 15 minutes. Centrifuge and supernatant were used for 5 minutes to analyze the AsA content (Kampfenkel *et al.*, 1995). Also, to measure the content of ascorbate, 0.2 ml of supernatant, 0.6 ml of sodium phosphate buffer (200 mM, pH=7.4), 0.2 ml of double-distilled water, 1 ml% 2.5 TCA, 0.8 ml H_3PO_4 (8.4%), 0.8 ml 2, 2 dipyridine (0.8%) were poured into a test tube. Then, $FeCl_3$ (0.3%) in the amount of 0.4 ml was added dropwise on the vortex to the test tube and placed in a hot water bath at 42°C for 40 minutes. The absorbance was then read at 525 nm. 2,2-Dipyridine was prepared instead of water in ethanol (70%) and distilled water was used as a control. To measure the content of total AsA in the test tube 0.2 ml of supernatant, 0.2 ml (dithiotreitol) DTT 0.5 mM, 0.4 ml of sodium phosphate buffer (pH 7.4, 0.02). 0 mM)

and the material inside the tube was mixed well and placed in a hot water bath of 42 degrees for 15 minutes. Then 0.2 ml (N-ethylmaleimid) NEM (0.025%) was added to the mixture and placed at laboratory temperature for 1 minute. The DHA content is also obtained from the difference between the total AsA content and the AsA content. AsA was prepared at concentrations of 0, 0.01, 0.03, 0.06, 0.12, 0.25, 0.5 and 1 mol/ml and then its standard graph was drawn. Finally, the results were reported as µmol/g fresh weight (Kampfenkel *et al.*, 1995).

DHA= Total AsA- AsA

Prepare cross-sections: The transverse cross-section was prepared manually from the ends of cut stems and the site of adventitious roots. After Manual cuts, the plant tissues were colored by double stain techniques. For this purpose, the sections were placed in sodium hypochlorite solution for 20 minutes. The slices were then washed with distilled water and 10% acetic acid was used for 2 minutes to eliminate the effect of bleach and soften the slices. After rinsing the slice with distilled water, staining was performed with Carmine dye solution and Methylene green (Vert méthylène) for 10 to 15 minutes. Finally, it was washed again with distilled water and finally, the stained sections were transferred to the slide for observation under a microscope.

Statistical analysis: In this study, SPSS 17.0 software was used for statistical analysis. Data were statistically analyzed by one-way analysis of variance and the means of all studied traits were compared using Duncan's test at a 5% probability level. Also, all charts were drawn using Excel software.

Results and Discussion

The effect of AsA on the induction and emergence of adventitious roots of Tradescantia cuttings: The effect of AsA on the induction of formation and number of emergence adventitious roots according to Figure 3 is significantly negative compared to the control. The results showed that the presence of AsA in the rooting environment of cuttings delayed the formation and appearance of new roots from the cut end of the stem. According to previous studies, vitamins are needed in small amounts for the normal growth and development of tissues in the plants. The presence of this group of substances in tissue culture medium for plant growth and development has been proven (Antonopoulou *et al.*, 2005). It has also been reported that different concentrations of AsA have different effects on plants. Low levels have a positive effect on the plant growth environment, while high levels adversely influenced cell growth and division (Deng *et al.*, 2012; Gallie, 2013). According to Figures 3 and 4, which show the effect of AsA on the number and length of adventitious roots, it can be seen that the decrease in adventitious root growth may be due to reduced mitotic division in meristematic regions and cell growth due to AsA (Smirnoff and Wheeler, 2000). Because AsA is a cofactor for the biosynthesis of several hormones, the results suggest that AsA affects the levels of the hormones auxin, cytokinin, and abscisic acid, as these hormones play an important role in rooting stimulation and development. To start the formation of adventitious roots, a certain concentration of natural growth regulators, especially auxin, is required in the plant. This seems to be caused by the accumulation of auxin at the bottom of the cuttings. The most prominent feature of auxins is their effect on the elongation and longitudinal growth of cells. The presence of AsA alone can reduce the function of cytokinin and auxin, although the mechanism and pathways describing this process are currently unclear (Sunitha, 2014).

The effect of AsA on the free amino acids content in adventitious roots of Tradescantia cuttings: The results of the effect of AsA on the free amino acid content of new roots according to Figure 5 showed that AsA significantly reduced the free amino acid content of roots compared to the control, Similar to the results

of AsA application on Carthamus tinctorius (Gadallah, 2000).

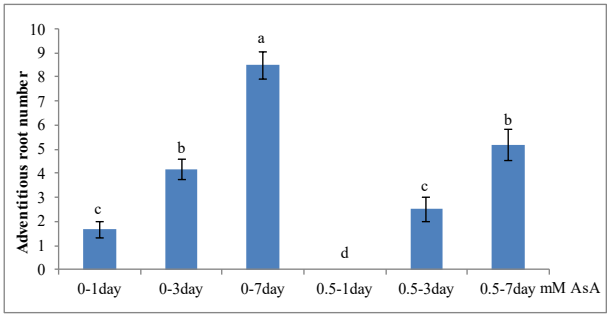

Figure 3. The effect of AsA on the number of adventitious roots

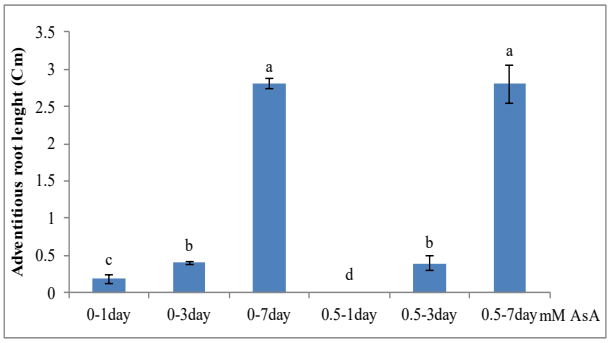

Figure 4. The effect of AsA on the length of adventitious roots

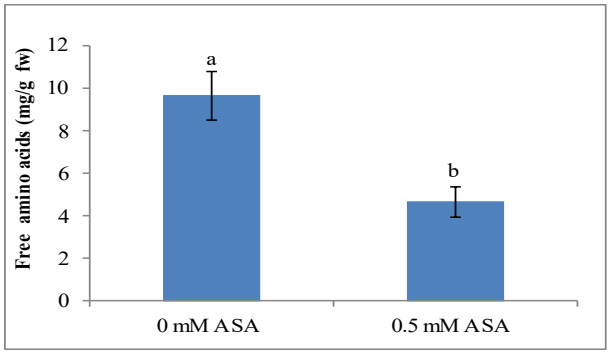

Figure 5. The effect of AsA on the free amino acid content of adventitious roots.

The results of this study indicate that AsA as substrate in the H_2O_2 detoxification and its conversion to water in the ascorbate-glutathione cycle has reduced the H_2O_2 content [29]. The amount of H_2O_2 produced in the cell indicates the balance between the production and decomposition of ROS in the cell. Since a high concentration of H_2O_2 is considered a cause of oxidative stress, one of the effects of AsA is the reduction of ROS production and subsequent production of free radicals and H_2O_2 [30, 31]. The main sources of H_2O_2 production in plant cells include electron transfer chain reactions in chloroplasts and mitochondria, Mehler reactions, peroxidase, and NADPH oxidase activity. When oxygen accepts an electron, it is converted to a superoxide radical, which is highly unstable and reacts with protons spontaneously or by superoxide dismutase to convert to H_2O_2. It is also produced through the reduction of superoxide by reductants such as ascorbate, thiol, ferredoxin, and so on. Thus, H_2O_2 levels are directly related to the amount of superoxide radical production [32, 33].

Low H_2O_2 can be a result of increasing the amount of AsA and activity of ascorbate peroxidase (APX). So the higher content or activity of APX leads to more removal H_2O_2. Little amounts of H_2O_2 - intervening in lignin biosynthesis- can delay polymerization of xylogen monomers and lignification.

AsA may inhibit the biosynthesis of excess amino acids and reduce its content in plants may be due to its antioxidant properties. Amino acids play an important role in stimulating the rooting and root growth of plants. For example, the amino acid tryptophan is a precursor to the auxin and phenylalanine is the lignin precursor. So, amounts of amino acids can affect new root formation and growth. Singh and his coauthors in 2018, investigated the effect of cutting time and use of auxin alone or in combination with AsA at a concentration of 2% on rooting of lime (Citrus aurantifolia L.) cuttings.

They reported that early spring and the use of auxin with AsA was the best treatment to increase the rooting of cuttings (Singh *et al.*, 2018). Amino acids are small organic molecules and are present in all living cells. Plants, unlike other organisms, can make amino acids using photosynthetic elements (carbon, oxygen, hydrogen, and nitrogen) and some other elements during the photosynthesis process. Amino acids are the building blocks of proteins involved in the synthesis of secondary metabolic compounds that have multiple nutritional, hormonal, and physiological roles

and can enhance plant vegetative and reproductive activities. Although amino acids make up proteins, some of them are also present freely in the cell. The concentration of these compounds is relatively low. However, protein synthesis decreases when the plant is exposed to dehydration or salinity stress. Therefore, some free amino acids, especially Proline, may accumulate in the plant and their concentration is very high. Amino acids are the most important metal-binding ligands in mM concentrations in plant tissues (Pohlmeier, 2004).

The effect of AsA on the H_2O_2 content in adventitious roots of Tradescantia cuttings: The effect of AsA on the H_2O_2 content of the new roots illustrated in Figure 6 showed a significant reduction in H_2O_2 content. In line with our results, a decrease in H_2O_2 content has been reported in wheat plants treated with AsA (Azzedine *et al.*, 2011).

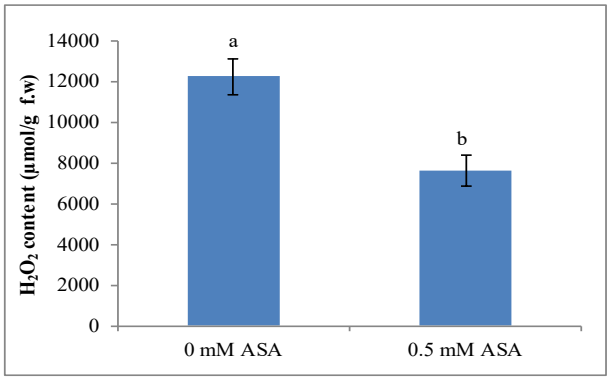

Figure 6. The effect of AsA on the H_2O_2 content of adventitious roots.

The effect of AsA on the APX activity in adventitious roots of Tradescantia cuttings: Application of AsA increased APX activity (Figure 7). Plants have enzymatic and non-enzymatic antioxidant systems to reduce ROS damage. APX is part of the enzymatic antioxidant defense system, which is a key enzyme in ROS scavenging. The APX plays an effective role in H_2O_2 deletion in the Mehler reaction or glutathione-ascorbate cycle (Mittler, 2002). The results indicate that in the glutathione-ascorbate cycle with the activity of ascorbate-peroxidase, ascorbate is oxidized to monohydro-ascorbate and therefore its amount in the plant has increased. It is also necessary to continue the ascorbate production cycle. For this purpose, the monohydro-ascorbate reductase, DHA reductase, and glutathione reductase are likely to be active in this cycle, using NADPH and glutathione to reduce ascorbate (Roychoudhury and Basu, 2012).

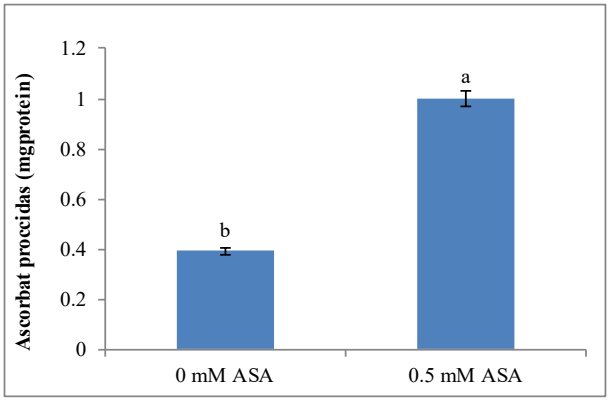

Figure 7. The effect of AsA on the APX activity of adventitious roots

The APX is one of the most important antioxidants that break down H_2O_2 into water and oxygen molecules [35]. APX, with its high affinity to H_2O_2, can help relieve plant poisoning. While other antioxidant enzymes can speed up this reaction, they have little affinity to H_2O_2 and cannot control as well as APX. The APX can regulate H_2O_2 as a signal molecule (Roychoudhury and Basu, 2012).

APX is activated in response to some of the environmental stresses such as salinity, drought, cold, microelement deficiency, iron stress, cold, polluted air, high light, and UV (Bonifacio *et al.*, 2011). Under stress, the increase in APX activity is due to the increase in ROS, which increases the expression of antioxidant enzyme genes and increases the activity of these enzymes by activating signal transduction pathways (Bonifacio *et al.*, 2011).

The effect of AsA on the AsA / DHA ratio in adventitious roots of Tradescantia cuttings: Due to the importance of AsA / DHA ratio, the content of both substances was measured, and the ratio of AsA / DHA was calculated and

presented in Figure 8. The results showed that AsA significantly reduced the ratio of AsA / DHA in the new roots compared to the control.

Since this ratio has decreased in this study, it can be inferred that alteration in ascorbate-glutathione cycle reactions leads to the accumulation of DHA. The decrease in this ratio is also related to the decrease in growth because the higher amount of this ratio leads to elongation and cell wall expansion. Apoplastic glutathione is low so DHA cannot reduce and converts to AsA and remains trapped in the apoplast. It is also reasonable with the concentration of external AsA to increase the total ascorbate, which contains all the compounds related to AsA. AsA recirculation in chloroplasts is a mechanism for regulating electron transfer. AsA is very sensitive to changes in environmental conditions. This vitamin acts as a cofactor for the APX, which is involved in the breakdown of H_2O_2. As the activity of APX increased, the content of AsA decreased and the content of total ascorbate and DHA increased. The content of AsA in the plant is largely dependent on the activity of the APX. Decreases in AsA indicate that the glutathione-ascorbate cycle has shifted toward DHA production. This enzyme oxidizes AsA to DHA [35]. Concentrations and ratios of ascorbate to DHA in apoplasts probably regulate cell growth and division (Horemans *et al.*, 2000). However, ascorbate consumption is almost the same during cell division and growth, but AsA/MDHA ratio during cell division is 6 to 10 times and during cell growth is 1 to 3 times more, which indicates that the reduced state of ascorbate is required for cell division. Ascorbate affects callus formation and mitosis (Hossain *et al.*, 2017).

The effect of AsA on the anatomical events during adventitious roots formation in Tradescantia cuttings: Figure 9 shows the results of photographing the cross-sections of T. fluminensis cuttings, which include the stages of formation of adventitious roots. The results showed that AsA delayed the emergence of rooting.

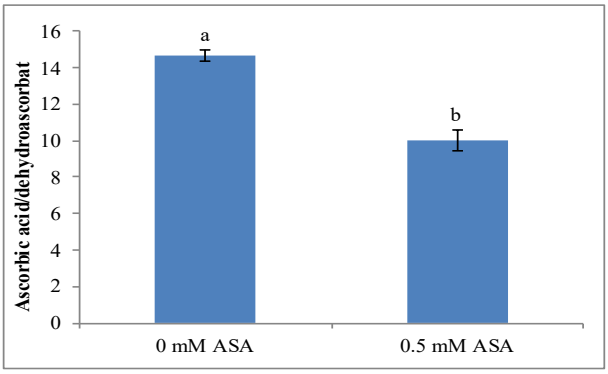

Figure 8. The effect of AsA on the AsA/DHA activity of adventitious roots

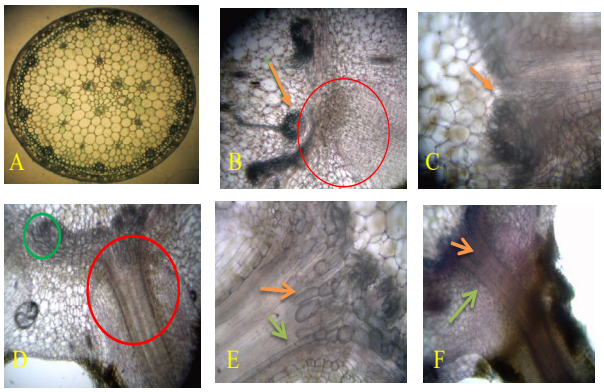

Figure 9. Stages of formation of adventitious roots from *T. fluminensis* cuttings, A) Overview of the cross-section of *T. fluminensis* stem with scattered vascular bundles (typical of monocots), B) Vascular bundle of the stem (Orange arrow) and primordium of adventitious root (Red Circle), C) De-differentiation and Transformation of parenchymal cells into tracheids (Orange arrow), D) Elongation zone of newly formed adventitious root (Red Circle) and Vascular bundle of mother plant (Green Circle) , E) Reticulate tracheid (Orange arrow) and Annular and spiral tracheid (Green arrow), F) Longitudinal section of newly formed adventitious roots Reticulate tracheid (Orange arrow) and annular and spiral tracheid (Green arrow).

As shown in Figure 9, the *T. fluminensis* is a monocot plant and the stem structure is the same as all monocotyledons. Many vascular bundles are located in scattered forms among ground tissue. The surrounding epidermis is green and has photosynthetic cells. Due to the double staining of the stem sections by Carmen (cellulose detector) and Vert méthylène (lignin detector), the parenchymal cell walls and phloem are seen as red because the primary cell

wall of these cells has cellulose (red with Carmen). However, the tracheid is green due to the lignification of the secondary cell wall.

The parenchyma cells of the vascular bundles show the first signs of differentiation and transformation into reticular tracheid (Figure 9 C and E). The differentiation of parenchymal cells is clear (Figure 9 C). It seems that in addition to the formation of tracheid to emergence adventitious roots from the stem and participate in water and nutrients uptake, internal changes take place to connect the stem vascular system to the new roots and produce an integrated system for efficient transfer of water and ions to the shoots (9 B). Also in Figure 9 E, after the formation of new roots and emergence of the stem, the annular and spiral tracheid can be recognized. Around the spiral tracheid, the cells elongate, which is a characteristic feature of the elongation zone of the root meristem. Although in this area the cells are still indistinguishable, their walls grow in two directions and become very long. This area is called the elongation zone. The area above this area is where the cells begin to differentiate (9 D).

The AsA molecule, as an antioxidant, plays a protective role in plant cells, and all higher plants synthesize and accumulate large amounts of this molecule. Its function as a major redox and cofactor of important enzymes, including enzymes involved in the regulation of photosynthesis, hormone biosynthesis, and reproduction of other antioxidants, is accepted. AsA regulates cell division and growth and is involved in signal transduction. Therefore, as previous studies have shown, different concentrations of AsA have different effects on plants. Low doses have positive effects on plant growth, while high doses seem to have negative effects on cell growth and division by complete removal of hydrogen peroxide, a very important signaling molecule (Hossain *et al.*, 2017).

It seems the AsA concentration used in this project was more than the required amount to stimulate cell division and adventitious root formation. The cut branches of *T. fluminensis* contain amounts of AsA, and the use of 0.5 mM of AsA delayed the formation of adventitious roots and slowed the elongation (Cell enlargement) of formed roots. Plant cell wall expresses monoamine oxidases (MAOs) that catalyze the oxidation of secreted amines and produce H_2O_2 in the process. The H_2O_2, so produced is used by cell wall peroxidases for lignification of the cell wall or plant defense (Verma and Sharma, 2010).

Conclusion

AsA plays an important role in many aspects of control not only of the cellular redox state and anti-oxidative activity in plant cells, but also of cell division and cell expansion, and plant development and growth as an enzyme cofactor. In general, it can be said that AsA has a positive effect and DHA impairs cell expansion and division. In this study, AsA delays the differentiation and formation of a new tracheid for rooting. This response is to increase the activity of APX and decrease the concentration of H_2O_2 and the effect on lignin biosynthesis. During tracheid differentiation and secondary cell wall lignification, lignin precursor free amino acids such as phenylalanine and H_2O_2 are required. According to the results, a decrease in free amino acid content and enhanced APX activity, limit lignin biosynthesis. Also, the precondition for the formation of the meristematic zone and the beginning of adventitious root growth and re-differentiation into tracheid cells depends on the amount of H_2O_2 and free amino acid, which AsA has delayed the process of lignification of cell walls and adventitious roots formation and emergence delays.

Acknowledgments

Thanks to the financial support of the Azarbaijan University of Tarbiat Moallem.

References

Antonopoulou, C., Dimassi, K., Therios, I., Chatzissavvidis, C. and Tsirakoglou, V. 2005. Inhibitory effects of riboflavin (vitamin b2) on the in vitro rooting and nutrient concentration of explants of peach rootstock gf 677 (prunus amygdalus× p. Persica). Scientia horticulturae, 106(2): 268-272.

Azzedine, F., Gherroucha, H. and Baka, M. 2011. Improvement of salt tolerance in durum wheat by ascorbic acid application. J. Stress Physiol. Biochem, 7(1): 27-37.

Bonifacio, A., Martins, M.O., Ribeiro, C.W., Fontenele, A.V., Carvalho, F.E., MARGIS-PINHEIRO, M. and Silveira, J.A. 2011. Role of peroxidases in the compensation of cytosolic ascorbate peroxidase knockdown in rice plants under abiotic stress. Plant, cell & environment, 34(10): 1705-1722.

Chaparzadeh, N., D'Amico, M.L., Khavari-Nejad, R.-A., Izzo, R. and Navari-Izzo, F. 2004. Antioxidative responses of calendula officinalis under salinity conditions. Plant Physiology and Biochemistry, 42(9): 695-701.

Davey, M.W., Montagu, M.V., Inze, D., Sanmartin, M., Kanellis, A., Smirnoff, N., Benzie, I.J.J., Strain, J.J., Favell, D. and Fletcher, J. 2000. Plant l-ascorbic acid: Chemistry, function, metabolism, bioavailability and effects of processing. Journal of the Science of Food and Agriculture, 80(7): 825-860.

De Cabo, R., González-Reyes, J., Cordoba, F. and Navas, P. 1996. Rooting hastened in onions by ascorbate and ascorbate free radical. Journal of Plant Growth Regulation, 15(2): 53-56.

Deng, X.-P., Cheng, Y.-J., Wu, X.-B., Kwak, S.-S., Chen, W. and Eneji, A.E. 2012. Exogenous hydrogen peroxide positively influences root growth and exogenous hydrogen peroxide positively influences root growth and metabolism in leaves of sweet potato seedlings. Australian Journal of Crop Science, 6(11): 1572.

Gadallah, M. 2000. Effects of acid mist and ascorbic acid treatment on the growth, stability of leaf membranes, chlorophyll content and some mineral elements of carthamus tinctorius, the safflower. Water, air, and soil pollution, 118(3): 311-327.

Gallie, D.R. 2013. L-ascorbic acid: A multifunctional molecule supporting plant growth and development. Scientifica, 2013.

Garrod, B., Lewis, B., Brittain, M. and Davies, W. 1982. Studies on the contribution of lignin and suberin to the impedance of wounded carrot root tissue to fungal invasion. New Phytologist, 90(1): 99-108.

Harding, V.J. and MacLean, R.M. 1916. A colorimetric method for the estimation of amino-acid α-nitrogen. Ii: Application to the hydrolysis of proteins by pancreatic enzymes. Journal of Biological Chemistry, 24(4): 503-517.

Horemans, N., Foyer, C.H. and Asard, H. 2000. Transport and action of ascorbate at the plant plasma membrane. Trends in plant science, 5(6): 263-267.

Horemans, N., Foyer, C.H., Potters, G. and Asard, H. 2000. Ascorbate function and associated transport systems in plants. Plant physiology and biochemistry, 38(7-8): 531-540.

Hossain, M.A., Munné-Bosch, S., Burritt, D.J., Diaz-Vivancos, P., Fujita, M. and Lorence, A. 2017. Ascorbic acid in plant growth, development and stress tolerance. Springer.

Jimenez, A., Hernandez, J.A., del Río, L.A. and Sevilla, F. 1997. Evidence for the presence of the ascorbate-glutathione cycle in mitochondria and peroxisomes of pea leaves. Plant physiology, 114(1): 275-284.

Kampfenkel, K., Vanmontagu, M. and Inzé, D. 1995. Extraction and determination of ascorbate and dehydroascorbate from plant tissue. Analytical biochemistry, 225(1): 165-167.

Matic, D. 2014. The function and metabolism of ascorbic acid in plants. Bachelor's Thesis, University of Zagreb, Zagreb, Croatia.

Mittler, R. 2002. Oxidative stress, antioxidants and stress tolerance. Trends in plant science, 7(9): 405-410.

Noctor, G. and Foyer, C.H. 1998. Ascorbate and glutathione: Keeping active oxygen under control. Annual review of plant biology, 49(1): 249-279.

Padh, H. 1990. Cellular functions of ascorbic acid. Biochemistry and Cell Biology, 68(10): 1166-1173.

Pastori, G.M., Kiddle, G., Antoniw, J., Bernard, S., Veljovic-Jovanovic, S., Verrier, P.J., Noctor, G. and Foyer, C.H. 2003. Leaf vitamin c contents modulate plant defense transcripts and regulate genes that control development through hormone signaling. The Plant Cell, 15(4): 939-951.

Peter, C.R. and Burdick, D.M. 2010. Can plant competition and diversity reduce the growth and survival of exotic phragmites australis invading a tidal marsh? Estuaries and Coasts, 33(5): 1225-1236.

Pignocchi, C., Fletcher, J.M., Wilkinson, J.E., Barnes, J.D. and Foyer, C.H. 2003. The function of

ascorbate oxidase in tobacco. Plant Physiology, 132(3): 1631-1641.

Pohlmeier, A. 2004. Metal speciation, chelation and complexing ligands in plants. In: Heavy metal stress in plants. Springer: pp: 28-46.

Qian, C., He, Z., Zhao, Y., Mi, H., Chen, X. and Mao, L. 2013. Maturity-dependent chilling tolerance regulated by the antioxidative capacity in postharvest cucumber (cucumis sativus l.) fruits. Journal of the Science of Food and Agriculture, 93(3): 626-633.

Roychoudhury, A. and Basu, S. 2012. Ascorbate-glutathione and plant tolerance to various abiotic stresses. Oxidative stress in plants: causes, consequences and tolerance. IK International Publishers, New Delhi: 177-258.

Seitz, J.C. and Clark, M.W. 2016. Identification, biology, and control of small-leaf spiderwort (tradescantia fluminensis): A widely introduced invasive plant. Document SL428. Department of Soil and Water Science, UF/IFAS Extention.

Singh, K.K., Krishan, C. and Singh, K. 2018. Propagation of citrus species through cutting: A review. Journal of Medicinal Plants Studies, 6(1): 167-172.

Smirnoff, N. 2011. Vitamin c: The metabolism and functions of ascorbic acid in plants. In: Advances in botanical research. Elsevier: pp: 107-177.

Smirnoff, N. and Wheeler, G.L. 2000. Ascorbic acid in plants: Biosynthesis and function. Critical reviews in plant sciences, 19(4): 267-290.

Sunitha, S. 2014. Regenerative effect of l-ascorbic acid on the in vitro grown plants. British Biotechnology Journal, 4(12): 1238-1252.

Verma, G. and Sharma, S. 2010. Role of h 2 o 2 and cell wall monoamine oxidases in germination of vigna radiata seeds.

Wang, W., Vinocur, B. and Altman, A. 2003. Plant responses to drought, salinity and extreme temperatures: Towards genetic engineering for stress tolerance. Planta, 218(1): 1-14.