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EFFECT OF ASCORBIC ACID CONCENTRATION ON STRUCTURAL CHARACTERISTICS OF APPICAL MERISTEMS ON *in vitro Aloe barbadensis* Mill.

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Abstract. Ascorbic acid is one of the major metabolite in higher plants cells which is known as effective factor when the cells enter to "S" phase from "G_1" phase of cytokinesis. This metabolite has antioxidant activity and increases plant tolerance against stressors such as salinity, pathogens, ozone, UV rays, etc. The current study used the common cellular and histological methods to evaluate the effect of 0.05 to 2.5 mM ascorbic acid on vegetative meristems of *Aloe barbadensis* plants obtained from stem explants propagated *in vitro* culture conditions. Results showed that low concentrations of ascorbic acid (0.5 to 1 mM) increase mitotic index in apical meristem and root quiescent center (QC). Moreover, treatment with ascorbic acid increases cellular dimensions in cell elongation region of root and mitotic divisions in this region. In some measurements, it was clear that in addition to increase root length in plants treated with ascorbic acid, distance from root hairs zone to root cap increases compared to the control, which is a logical conclusion from increasing cell elongation and divisions in cell elongation zone. Also, ascorbic acid increased production of secondary roots through stimulating cells of pericycle and increasing divisions in this region. Apical meristem of stem treated with ascorbic acid had more convexity homogenous with more chromophilic level. Increasing stem length and number of leaves in plants treated with ascorbic acid could be related to the high cells' mitotic activity in stem apical meristem. Moreover, ascorbic acid could stimulate cell division, increasing area of meristem zone, and effective on severity of differentiation.

Key words: aloe, cell division, differentiation, histology, shoots and roots apical meristem

INTRODUCTION

Ascorbic acid is one of the best famous antioxidants existing in plants which protect plant's cells from oxidative harms [Noctor and Foyer 1998]. Ascorbic acid has various functions in plant physiological processes. This metabolite acts as electron donor in different biosynthetic pathways [Arrigoni and de Tullio 2000] and regulates cell elonga-

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tion and cell multiplication [Gonzalez-Reyes et al. 1998]. Researches have been conducted in two recent decades concentrated on ascorbic acid's function in regulating plant cell multiplication [Arrigoni et al. 1992, Wang and Faust 1992, de Gara et al. 1996, Tommasi et al. 1999]. These researches show that ascorbic acid influences on cell elongation and multiplication [Horemans et al. 2000]. In addition, ascorbic acid is one of the important factors to regulate passing cell through cell cycle [Potters et al. 2002]. Dehydroascorbate (DHA), an oxidized molecule, is a redox-active related to development of cell cycle. Potters et al. [2000] have shown that DHA revival is capable to influence on normal development of plant cell cycle. Articles published until now support this theory that changes in rate of ascorbic acid to intracellular DHA should be effective on the cell cycle. These results have been achieved through survey on development of cell cycle after adding DHA and ascorbic acid to culture of tobacco synchronized suspension cells (BY-2) [Nagata et al. 1992]. Also, histo-chemical detection of ascorbic acid in apex of maize's root showed that root quiescent center's cells have low or unrecognizable level of ascorbic acid which is likely due to high level of this acid in quiescent center [Nancy et al. 1995]. As ascorbic acid is a required compound for passing cell through "G_1" to "S" phase in cell cycle, its low level in quiescent center may be responsible for maintenance and retention of cells with low division in "G_1" phase which are found majorly in that phase [Nancy et al. 1995]. We investigated the effect of ascorbic acid on structural characteristics of root and shoot apical meristems of *A. barbadensis in vitro* plantlets.

MATERIALS AND METHODS

Plant material. Stem cutting of *A. barbadensis* was obtained from the mother plants free of symptoms of disease and pest problem growing in a commercial greenhouse, located in Mahallat city (N 53°23' and S 50°30'; altitude, 1747 m above sea level), Iran. Cuttings were washed thoroughly under running tap water for 30 min. Stems with buds were surface sterilized with 20% (w/v) NaOCl for 15 min followed by three rinses with sterile distilled water for 30 min. The surface disinfected stems were cut into 5–7 mm segments of shoot tips and internodes as explants.

Culture media. Explants were cultured in Petri dishes containing basal MS [Murashige and Skoog 1962] medium supplemented with plant growth regulators, KIN and IAA, both with concentration of 1 mg l^{-1}. Sucrose (3%) was used as carbon source and media were solidified with Agar-agar (0.7%). The pH was adjusted to 5.7 prior to autoclaving at 121°C and 102 kpa for 20 min. Ascorbic acid were dissolved in distilled water and sterilized by membrane filtration (0.22 μm) and added to the sterilized medium. Five explants were inoculated in each Petri dishes and three replicates were taken.

Growth conditions. Cultures were kept in growth chamber under 16 h photoperiods with light intensity of 56 μmol m^{-2} s^{-1} provided by cool-white fluorescent tubes at 26 ±1°C and 70% relative humidity of the air. Length of roots and stems were measured weekly.

Observation and measurements. Regenerated seedlings were leaved from the medium and evaluated. Terminal buds of the stem and also a few millimeters of the end of

root were taken and surveyed through proper cellular and histological methods. The process of specimen preparation for anatomical investigation is as follows: 1. Fixation; 2. Tissue dehydration; 3. Infiltrating and embedding tissues; 4. Sectioning and mantling; 5. Staining [Ruzin 1999]. Different staining methods used in this research was to determine the meristematic cells conditions and tracking of ascorbic acid in the cells. Double staining (hematoxylin and eosin) was used to assess dividing cells. Histo-chemical method was used in order to determine of ascorbic acid in tissues, based on the ability of ascorbic acid for deposition of metallic silver from silver nitrate. Roots were put into the solution of silver nitrate (1%) and then they were prepared for tissue cuttings. Sections of apical shoots and roots were performed using a microtome.

RESULTS

Statistical analysis of the results showed that callus formation on explants in culture media increased as ascorbic acid concentration increased. This increase in callus induction was more in internodes explants than that of shoot tip explants. Maximum callus induction was induced on MS medium supplemented with 1 mM ascorbic acid. While maximum bud regeneration was obtained in medium supplemented with 0.5 mM ascorbic acid for both explants, and the most propagules in this concentration were related to stem internodes explants (fig. 1). On the other hand, ascorbic acid stimulates root formation and increased root growth.

Fig. 1. Callus induction and buds' regeneration in medium containing 0.5 mM ascorbic acid after 30 days growth periods

Effect of exogenous ascorbic acid on stem apical meristem. In areas with exogenous ascorbic acid, stem apical meristem had more convexity, with more chromophilic level, so that this chromophily is more homogenous, while in control, lateral areas of apical meristem have more chromophilic level than that of middle areas. Comparing stem's apical meristem in control plants and plants treated with 1 mM of ascorbic acid has been shown in Fig. 2.

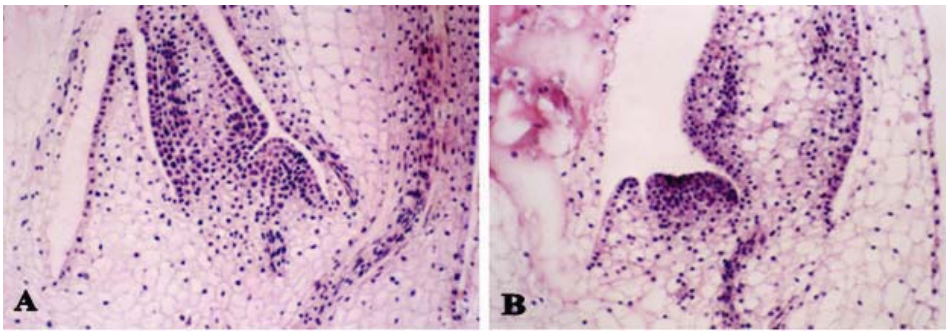

Fig. 2. A – apical meristem of stem in control sample, B – apical meristem of stem treated with 1 mM ascorbic acid. Samples were stained with hematoxylin-eosin (magnification of A and B: 130×)

Effect of exogenous ascorbic acid on root apical meristem. Different staining methods was applied to determine the cells conditions and tracking of ascorbic acid in the cells. Double staining was used to assess dividing cells (figs 3A, B and C). Histochemical method was used to determine of ascorbic acid in the cells (figs. 4A, B, C and D). In survey about apical area in root of Aloe plants grown in medium without exogenous ascorbic acid and comparing them with roots treated with 0.5 and 1 mM ascorbic acid, the following differences could be mentioned briefly: (1) Compared to the control, root cap in plants treated with 0.5 and 1 mM ascorbic acid, became more squeezed and shorter, (2) Root diameter increased in roots treated with ascorbic acid, in comparison with control, (3) Due to more cell division, parenchyma cells of root cortex were more squeezed and more arranged than that of control samples, (4) Differentiation of central column in plants treated with ascorbic acid occurred sooner and generated earlier than that of control plants, (5) Compared to the control samples, quiescent center of roots in samples treated with ascorbic acid became more active and dividing cells were observed on them, (6) Generally, numbers of dividing cells in samples treated with ascorbic acid were more abundant than that of control samples and (7) In cell elongation zone, ascorbic acid increased cells' size and number (figs 3 and 4).

The use of histochemical method for determining the presence of ascorbic acid. In order to determine the existence of ascorbic acid in tissues, histochemical method was used based on ability of ascorbic acid in creating deposition of metallic silver from silver nitrate. Although, this technique is not specialized for ascorbic acid, but this experiment shows better results in low temperature and pH, when other reducing agents are not active. Incubated roots in 0.5 mM ascorbic acid for 48 h showed increasing of cell

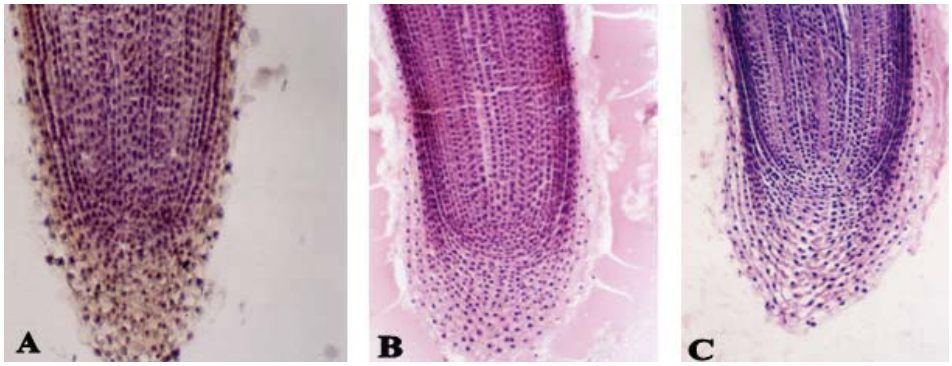

Fig. 3. Longitudinal sections of aloe root apex: A – control sample, B – sample treated with 0.5 mM ascorbic acid, C – sample treated with 1 mM ascorbic acid. Samples were stained with hematoxylin-eosin (magnification of A, B and C: 100×).

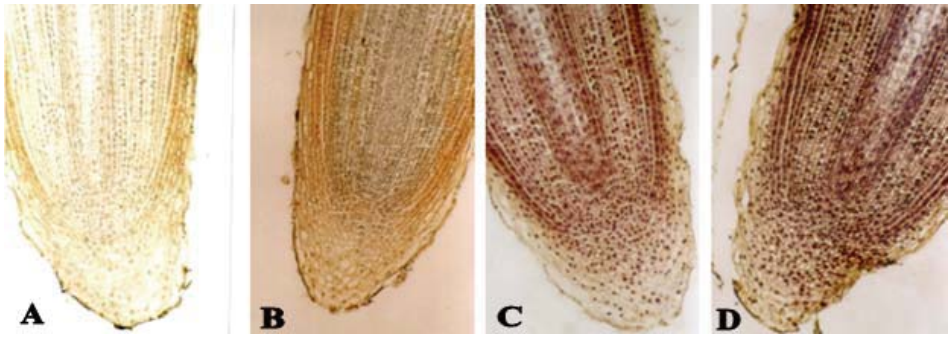

Fig. 4. Longitudinal sections of aloe root apex: A – control sample, stained with silver nitrate, B – sample treated with 0.5 mM ascorbic acid, stained with silver nitrate, C – control sample, stained with silver nitrate and hematoxylin-eosin, D – sample treated with 0.5 mM ascorbic acid, stained with silver nitrate and hematoxylin-eosin (magnification of A, B, C and D: 100×)

division activity in comparison with incubated roots in water. As it has shown in longitudinal section of root apex (fig. 4), the most prominent case is activation of cells in quiescent center. High chromophilic level of the core which is sign of DNA replication shows that the cells have passed through G_1 phase to S phase during treatment period. Relatively chromatic stains could be observed in peri-vascular areas in comparison with parenchyma zone which explain that concentration of ascorbic acid is considerably more in cytosol than in vacuole and high chromophilic level of meristematic cells could be seen in this method. Also, chromophilic level of root quiescent center in treated samples is more than that of control samples which shows more concentration of ascorbic acid in this zone.

DISCUSSION

Ascorbic acid effect plant growth and increased activity of apical meristem. This increase in growth may due to include from increase in mitotic division in meristematic zones and cell growth in length due to ascorbic acid influence [Smirnoff et al. 2000]. Obtained results confirmed that ascorbic acid oxidizes to DHA after it is added to medium and then it is absorbed and reduced to ascorbate inside the cells [Potters et al. 2000]. The current results confirmed that activity of ascorbic acid oxidase could be found in cell wall and many cellular compartments, produces ascorbate free radicals (AFR) and DHA. Through hyperpolarization of membrane, AFR stimulates cell growth and therefore, ion absorption and enlargement of vacuoles occur [Hindalgo et al. 1989, Cordoba and Gonzalez-Reyes 1994]. DHA causes flexibility in cell wall through preventing lateral connections between structural proteins and hemicellulose. On the other hand, produced oxalic acid through DHA, increases cell wall loosening by reducing the numbers of calcium bridges between pectin chains [Lin and Varner 1991]. This is similar to our finding which indicated that supplemented of ascorbic acid to medium increased length of cells in cell elongation zone of root and also increased distance between root hairs zone and root tip.

In provided tissue sections from root apex in control samples and samples treated with ascorbic acid, numbers of dividing cells were observed in roots quiescent center treated with ascorbic acid. On the other words, ascorbic acid stimulates cells of this region and induces cell division. These results are in agreement with those obtained by Liso et al. [1984] and Kerk and Feldman [1995] on root of *Allium cepa* L. and *Zea mays* L. Current research demonstrated that ascorbic acid in medium increases its concentration in QC and therefore, mitotic index increases in this region. Also, in tissue sections obtained from root apex, dividing cells and chromophilic level of this region's cells were clearly higher than those of control samples. Based on the current research, it is concluded that ascorbic acid has a similar activity with auxin. Auxin is known as a rooting hormone in proper concentration. Ascorbic acid has a delight effect on rooting, too.

CONCLUSIONS

1. Ascorbic acid effects on mitotic index. The 0.5 and 1 mM ascorbic acid increase mitotic index in apical meristem and root quiescent center (QC).
2. The use of ascorbic acid increase cells dimensions and mitotic divisions.
3. Ascorbic acid increase root length and distance from root hairs zone to root cap.

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WPŁYW STĘŻENIA KWASU ASKORBINOWEGO NA STRUKTURALNE CECHY MERYSTEMU WIERCHOŁKOWEGO W KULTURZE *in vitro* *Aloe barbadensis* Mill.

Streszczenie. Kwas askorbinowy jest jednym z głównych metabolitów w wyższych komórkach roślin i istotnym współczynnikiem efektywności, gdy komórki wchodzą w fazę „S" z fazy „G_1" cytokinezy. Metabolit ten ma właściwości utleniające oraz zwiększa tolerancję roślin na stresory, takie jak zasolenie, patogeny, ozon, promienie UV itd. W niniejszym badaniu wykorzystano powszechnie znane metody komórkowe i histologiczne. Oceniano wpływ 0,05 do 2,5 mM kwasu askorbinowego na wegetatywne merystemy *Aloe barbadensis* uzyskane z eksplantów łodyg rozmnożonych w warunkach hodowli *in vitro*. Uzyskane wyniki pokazały, że niskie stężenia kwasu askorbinowego (0,5 do 1 mM) zwiększają wskaźnik mitotyczny w merystemie wierzchołkowym oraz centrum spoczynkowym korzeni (QC). Poza tym zabieg kwasem askorbinowym zwiększa wymiary komórkowe w rejonie elongacji komórek korzenia oraz podziały mitotyczne w tym rejonie. W niektórych pomiarach jasne było, iż poza zwiększeniem długości korzenia roślin traktowanych kwasem askorbinowym, zwiększa się odległość od strefy włośników do czapeczki korzeniowej w porównaniu z kontrolą, co jest logiczną konsekwencją zwiększenia elongacji komórek i podziałów w strefie elongacji komórek. Kwas askorbinowy zwiększał także wytwarzanie wtórnych korzeni poprzez stymulowanie komórek perycyklu oraz zwiększanie podziałów w tym rejonie. Merystem apikalny traktowany kwasem askorbinowym miał większą wypukłość homogeniczną z poziomem chromofilowym. Zwiększenie długości łodygi oraz liczby liści u roślin traktowanych kwasem askorbinowym mogło być związane z wysoką aktywnością mitotyczną komórek w merystemie apikalnym łodygi. Ponadto kwas askorbinowy, wpływając na intensywność zróżnicowania, mógł stymulować podział komórek poprzez zwiększenie obszaru stref merystemu.

Słowa kluczowe: aloes, podział komórek, zróżnicowanie, histologia, apikalny merystem pędów i łodyg

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