Influence of ascorbic acid on growth and micropropagation of *Aloe barbadensis* Mill.

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Abstract

Ascorbic acid (AsA) plays various roles in plant metabolism. This study was carried out to evaluate the effect of different concentrations of AsA on the growth and micropropagation of *Aloe barbadensis* Mill., an important medicinal and ornamental herb, for the first time. In this regard, results obtained from applying different concentrations of AsA on variables such as aerial part length, root length and number, number of propagules, medium browning, and the fresh and dry weights of plants were analysed after 8 weeks. Control plants showed slower growth in the aerial parts compared to plants treated with AsA. Additionally, the leaves were smaller in the control plants. The fresh and dry weights of the aerial parts were less in the control plants than in those treated with AsA. There was a significant increase in the number of propagules produced in the different treatments compared to the control treatment. The average number and length of roots produced in plants treated with AsA were higher than those produced in the control plants. Furthermore, browning of the medium and tissue cultures was reduced in plants treated with AsA due to the presence of different phenolic compounds in these plants. Overall, AsA at a concentration of 80 mg L^{-1} had the greatest effect on the induction of growth and development of *A. barbadensis* Mill. in vitro.

Keywords: Aloe, medicinal plants, plant growth regulators, tissue culture, vegetative growth.

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Introduction

Aloe barbadensis Mill. belongs to the Liliaceae family and is an important medicinal and pharmaceutical plant. *A. barbadensis* has also been widely grown as an ornamental plant (Duyff, 2017). This species has been used in the food and cosmetic industries as well (Uikey et al., 2013). *Aloe* spp. contains various secondary metabolites. Gels prepared from *Aloe* leaves are significant due to their diverse applications in the medicinal and

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cosmetic industries. *Aloe* is mainly propagated by suckers and offshoots, which is too slow a process for commercial plant production. One of the most important and effective methods for the rapid vegetative propagation and breeding of lilies is in vitro culture. Successful in vitro propagation of lilies depends on many factors, such as the type and concentration of plant growth regulators (PGRs), particularly auxins and cytokinins, as well as the type of culture medium and (Kaviani et al., 2019; Taha et al., 2018; Youssef et al., 2019). The successful use of tissue culture techniques for the rapid propagation of some species of the genus *Aloe* has been reported (Hashemabadi and Kaviani, 2008; Uikey et al., 2013).

Ascorbic acid (AsA), commonly known as vitamin C, plays an essential role in plant resistance against biotic and abiotic stresses (Chen et al., 2021; Habibie et al., 2019; Lo'Ay and El-Khateeb, 2018; Martínez-Ortiz et al., 2019), mainly due to its antioxidant properties and its ability to provide a special redox status in symplastic and apoplastic compartments (del Carmen Córdoba-Pedregosa et al., 2003). At the intracellular level, AsA is involved in cell division and multiplication, while at the extracellular level, it is important for defense against pathogens and regulation of cell elongation. In both cases, AsA is considered a substrate for enzymes that regulate these processes, such as ascorbate peroxidase or other peroxidases that affect the cell wall (del Carmen Córdoba-Pedregosa et al., 2003). It has been proven that symplastic ascorbate is associated with cell division and multiplication (Horemans et al., 2000). Cell division is a fundamental biological process governed by molecular networks initiated in the apical meristems of plants. AsA is a crucial molecular modulator involved in cell proliferation (Kka et al., 2018). Generally, it is accepted that ascorbate facilitates cell elongation by inhibiting enzymes involved in strengthening the cell wall.

The primary method of non-sexual propagation for this plant is shoot production(Kaviani, 2014). This method is used for small-scale *Aloe* culture, but to cultivate it on a larger scale, a tissue culture system is necessary. There are no reports regarding the effect of AsA on the in vitro propagation of the Liliaceae family, particularly *Aloe* spp. Thus, this research, conducted for the first time, seeks to investigate the growth and micropropagation of *Aloe barbadensis* Mill. under in vitro conditions influenced by different concentrations of exogenous ascorbic acid.

Materials and Methods

Stem cuttings of *Aloe barbadensis* Mill. were obtained from plants free of disease symptoms and pest problems growing in a commercial greenhouse. Stem cuttings were washed 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. Explants were cultured in Petri dishes containing basal MS (Murashige and Skoog, 1962) medium supplemented with plant growth regulators, KIN and IAA, both at a concentration of 1 mg L-1, and different concentrations of AsA (0, 4, 10, 60, 80, 100, 200, and 500 mg L^{-1}). Sucrose (3%) was used as a carbon source, and the medium was 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. Five explants per jam jar were inoculated, and three replicates were prepared. Cultures were kept in a growth chamber under 16 h photoperiods with a light intensity of 2500 lux, provided by cool-white fluorescent tubes, at 26±1°C and 70% relative humidity.

Shoot length, root length, root number, propagule number, plant fresh weight, plant dry weight, and medium browning were evaluated. The lengths of roots and stems were measured weekly. Regenerated seedlings were removed from the medium and evaluated.

The experimental design was factorial with an R.C.B.D. design, carried out with unequal repetition. All experiments were carried out in five replicates. For statistical analysis, a complementary approach was used: ANOVA was performed, and means were compared using Tukey's test ($p<0.05$) in SAS software package, version 9.1. Data processing of the results was carried out in Excel.

Results

We studied the effect of different concentrations of ascorbic acid on the growth and micropropagation of *Aloe barbadensis* Mill., an ornamental and medicinal plant. The characteristics studied were shoot length, root length, root number, number of produced propagules, fresh weight, and dry weight. Our data revealed that there were differences in the effect of the different concentrations of ascorbic acid on these characteristics.

Effect of ascorbic acid on shoot length

The medium supplemented with 500 mg L^{-1} ascorbic acid resulted in the highest shoot length (11.00 mm) (Fig. I). The lowest shoot length (2.00 mm) was obtained in control plants. Data analysis showed that the effect of ascorbic acid was significant on the length of the shoot ($p \leq 0.01$). The average shoot length in plants treated with 10 mg L^{-1} of ascorbic acid was twice as much in comparison to control plants, and it showed a 1.95-fold increase in plants treated with 4 mg L^{-1} ascorbic acid. This increase was approximately 2.8 times more in plants treated with 60 mg L-1 ascorbic acid and 4.2 times more in plants treated with 200 mg L^{-1} ascorbic acid compared to the control plants. The largest increase in length was observed in plants treated with 500 mg L^{-1} ascorbic acid, which showed 5 times more growth in comparison with control plants ($P<0.05$). At concentrations higher than 500 mg L^{-1} ascorbic acid, growth decreased quickly (Fig I). The decrease in shoot length in plants treated with 80 mg L^{-1} ascorbic acid is likely due to the high percentage of produced propagules at this concentration. In addition, in media supplemented with various concentrations of ascorbic acid, plants had a natural appearance with green leaves during growth, while in media without ascorbic acid, the leaves of some samples turned yellow-brown.

Effect of ascorbic acid on root length

The greatest increase in root length (18.50 mm) was observed in plants treated with 10 mg L^{-1} ascorbic acid ($P<0.01$), which was 3 times more than the root length in control plants (Fig. I). The smallest increase in root length (5.80 and 6.00 mm) was observed in control plants and those treated with 600 mg L^{-1} ascorbic acid, respectively (Fig. I). Media with more than 20 mg L^{-1} ascorbic acid produced plants with thicker roots, and in media with more than 80 mg L^{-1} ascorbic acid, an increase in root thickness and broad, band-shaped roots was observed.

Effect of ascorbic acid on root number

The medium supplemented with 500 mg L^{-1} ascorbic acid resulted in the maximum root number (16.00) (Fig. II). The minimum root number (5.70) was obtained in control plants. Data analysis showed that the effect of ascorbic acid was significant on root number ($p \leq 0.01$). The difference in root number in plants treated with concentrations of 10 mg L^{-1} ascorbic acid and higher was significant ($P<0.05$) compared to control plants. The number of roots in plants treated with concentrations higher than 500 mg L^{-1} ascorbic acid decreased, and at concentrations above 600 mg L^{-1} ascorbic acid, rooting was inhibited. Additionally, if some roots emerged outside the culture medium conditions (ex vitro), their growth stopped.

Effect of ascorbic acid on the number of produced propagules

Our study on the effect of ascorbic acid on the number of produced propagules revealed that ascorbic acid had a significant effect on the number of propagules (Fig. II, III and IV). Statistical analysis showed that ascorbic acid had a significant effect on the number of produced propagules ($P \leq 0.01$). The maximum and minimum numbers of propagules (7.70 and 1.70) were observed on medium containing 80 mg L^{-1} ascorbic acid and in the control, respectively (Fig. II, III and IV). The number of propagules in plants treated with concentrations of 10 mg L^{-1} ascorbic acid and higher showed a significant difference ($p<0.05$) compared to control plants. This number increased gradually with higher concentrations, and the greatest increase in the number of propagules was observed at 80 mg L^{-1} ascorbic acid (approximately 4.7 times more compared to the

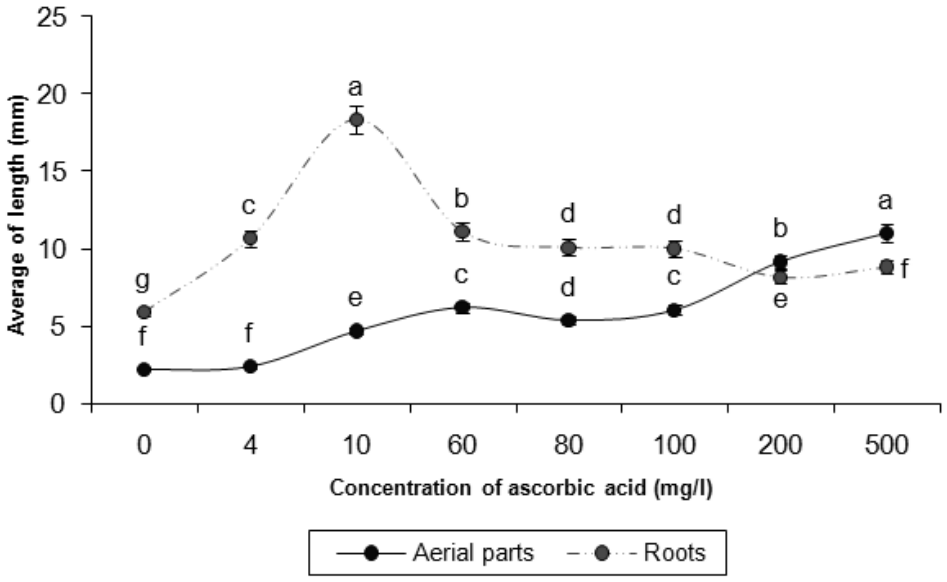

Fig. I. The effect of different concentrations of ascorbic acid on length of aerial parts and roots of *Aloe barbadensis* Mill. plantlets cultivated on MS medium supplemented with 1 mg L^{-1} IAA and KIN. Maximum length of aerial parts and roots was obtained at 500 and 10 mg L^{-1} ascorbic acid, respectively.

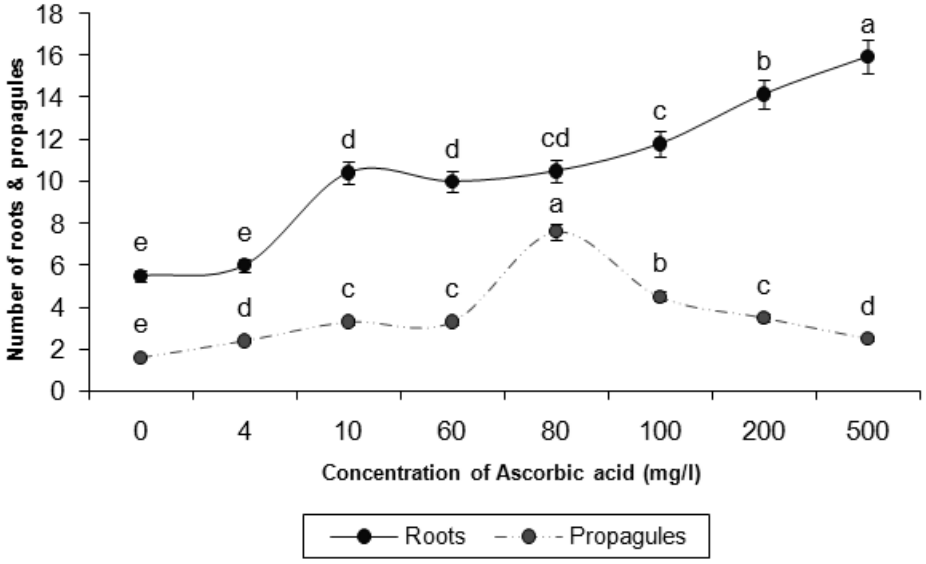

Fig. II. The effect of different concentrations of ascorbic acid on the number of propagules and roots of *Aloe barbadensis* Mill. plantlets cultivated on MS medium supplemented with 1 mg L^{-1} IAA and KIN. Maximum number of propagules and roots was observed at 80 and 500 mg L^{-1} ascorbic acid, respectively.

control) ($P<0.01$). At concentrations higher than 80 mg L^{-1}, the number of propagules decreased gradually (Fig. II). By selecting internodes as explants in the same culture conditions, the number of produced propagules could be increased to 19.8 propagules per explant in medium supplemented with 80 mg L^{-1} ascorbic acid.

Effect of ascorbic acid on fresh and dry weight of plants

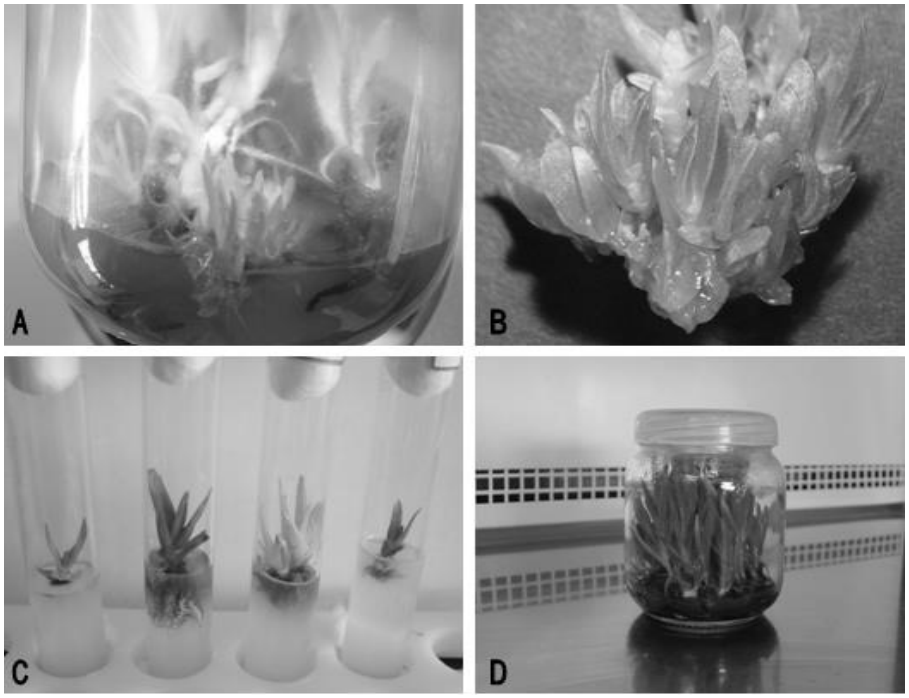

Fig. III. *In vitro* multiplication of *Aloe barbadensis*Mill., on MS medium supplemented with 1 mg L^{-1} IAA and KIN. A. Produced propagules from internode explants treated by 80 mgl^{-1} ascorbic acid. B. Callus mass and regenerated plants. C. Shooting and rooting responses *in vitro* of explants. From right to left: control treatment, 0, 80, 100 and 200 mg L^{-1} ascorbic acid. D. Explants treated with 80 mgl^{-1} ascorbic acid. The best shoot multiplication and growth was observed at 80 mg L^{-1} ascorbic acid.

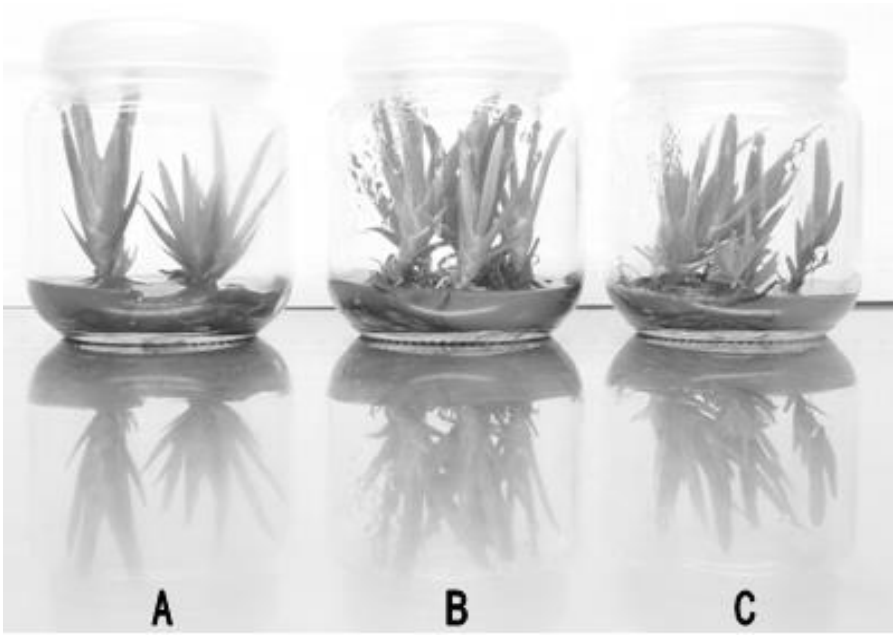

Fig. IV. Clonal propagation of *Aloe barbadensis* Mill., on MS medium supplemented with 1 mg L^{-1} IAA and KIN. Treated by 4 (A), 10 (B) and 20 (C) mg L^{-1} ascorbic acid. Highest multiplication rate was obtained in 80 mg L^{-1} ascorbic acid.

There was a significant difference ($P<0.01$) in the fresh and dry weights of plants treated with various concentrations of ascorbic acid compared to the control plants. The highest fresh and dry

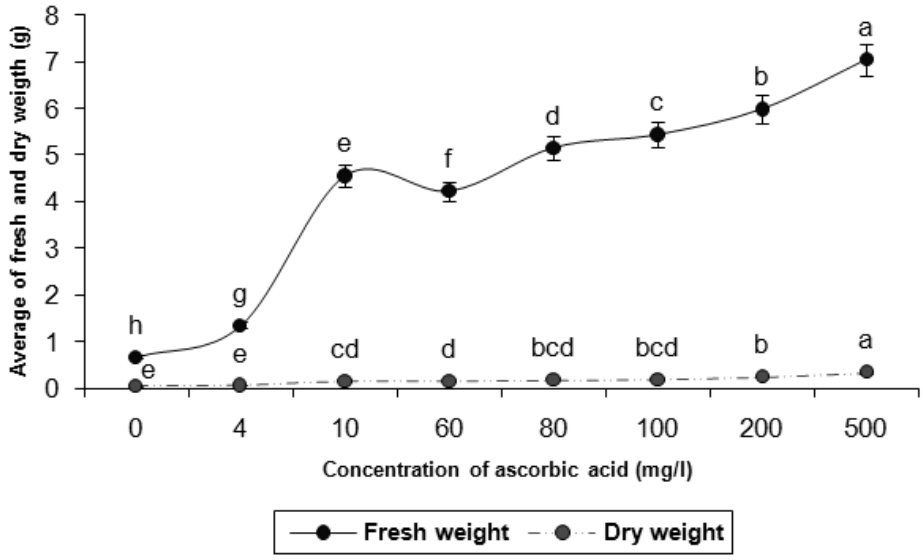

Fig. V. The effect of different concentrations of ascorbic acid on fresh and dry weights of *Aloe barbadensis* Mill. plantlets cultivated on MS medium supplemented with 1 mg L^{-1} IAA and KIN. Highest fresh and dry weights were observed at 500 mg L^{-1} ascorbic acid.

weights (1.7 and 2.3 g, respectively) were observed in plants treated with 500 mg L^{-1} ascorbic acid (Fig. V). The lowest fresh and dry weights (0.65 and 0.02 g) were observed in control plants and plants treated with 600 mg L^{-1} ascorbic acid, respectively (Fig. V). At concentrations higher than 500 mg L-1 ascorbic acid, the fresh and dry weights of the plants decreased quickly. At these concentrations, explants grew slowly, and a considerable decrease was seen in their fresh and dry weights. At concentrations higher than 600 mg L^{-1} ascorbic acid, the growth of samples stopped.

Effect of ascorbic acid on browning of plant tissues and media

Due to the presence of various phenolic compounds in *Aloe barbadensis* Mill., which are secreted into the medium, adding ascorbic acid as an antioxidant prevents browning of the medium and tissues, promoting better growth for the cultured plants.

Discussion

Some reports have shown the effect of PGRs, particularly auxins (NAA, IBA, 2,4-D, and IAA) and cytokinins (BA, Kin, and BAP), on the in vitro propagation of *Aloe* spp. (Hashemabadi and Kaviani, 2008; Niguse et al., 2020; Shibru et al., 2018; Singh et al., 2020; Uikey et al., 2013; Welehaweria and Sbhatu, 2023). The explants used for this purpose are mainly stem discs, leaf segments, shoot tips, lateral shoots, and offshoots.

There are no reports about the effect of ascorbic acid (AsA) on the in vitro micropropagation of the lily family, especially *Aloe* spp. Ascorbate regulates cell growth by controlling parameters such as the biosynthesis of proteins rich in hydroxyproline, which is required for development from the G1 to G2 phase in the cell cycle, lateral connections of cell wall glycoproteins and other polymers, and redox reactions in the plasma membrane, which are engaged in length-increase mechanisms (Córdoba and González-Reyes, 1994).

The free radical of ascorbate induces high vacuolization of cells for elongation. This effect could be related to redox system activity connected to the plasma membrane (Córdoba and González-Reyes, 1994). During the conducted experiments, it was observed that the emergence of roots in explants happens 3 to 4 days' sooner, and roots are longer and have more branches in plants treated with different concentrations of ascorbic acid than in control plants. Similar results

were reported in some other plants (Potters et al., 2000).

These researchers showed that AsA stimulates cell multiplication in all root meristems and the pericycle. The decrease in root length at high concentrations of AsA in the medium may be due to an increase in dehydroascorbate concentration produced from ascorbate oxidation in the medium. Potters et al. (2000) reported that, in contrast to the stimulating role of ascorbate, oxidized ascorbate (dehydroascorbate) prevents cell multiplication. Results obtained from these experiments could be consistent with the mentioned reports. It was previously shown that this form of oxidized ascorbate (dehydroascorbate) could be absorbed by plant cells (Horemans et al., 2000). The reduction of dehydroascorbate and its conversion to ascorbate in the aerial parts of plants provides a higher level of ascorbate for the apical meristem of the stem. Thus, it increases cell division and multiplication, leading to increased growth in aerial parts, as mentioned in this research. These findings are consistent with those reported by Horemans et al. (2000). In addition, the increase in fresh and dry weight reported in this study is directly related to the increase in ascorbate concentration in the medium, such that a decrease in ascorbic acid in the medium reduces biomass production. Furthermore, plant growth and development are controlled by plant growth regulators such as auxins, gibberellins, and abscisic acid.

Regarding the increase in the number of produced propagules and the highest number at a concentration of 80 mg L^{-1} ascorbic acid, it could be suggested that at this concentration, cell division is increased, leading to more callus production on the one hand, and on the other hand, more cells are differentiated, resulting in new seedlings. More studies on determining the concentration of ascorbic acid in cellular differentiation from callus mass in medium without ascorbic acid, and its comparison with produced calli under treatments, will explain this process.

Conclusion

Ascorbic acid (AsA), commonly known as vitamin C, is a crucial molecular modulator involved in cell proliferation. AsA plays positive roles in plants. This compound is also an antioxidant that absorbs phenolic compounds, especially from the culture medium. Various phenolic compounds in *A. barbadensis* Mill. are secreted into the medium. Adding AsA to the medium prevents the medium and tissues from turning brown, and it also induces more proper growth for cultured plants. AsA at a concentration of 80 mg L^{-1} had the highest effect on shoot multiplication and root number of *A. barbadensis* Mill. in vitro.

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