Effect of Antioxidant (Ascorbic Acid, AsA) on Maize (Zea mays L.) Growth

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ABSTRACT: Ascorbic acid (AsA), an antioxidant form of vitamin C, helps the plants in photosynthesis as an enzyme's cofactor. It also increases the rate at which plants grow, produce, and germinate. The main goal of this study was to find out how ascorbic acid affects the growth of maize (*Zea mays* L.) while applying on rooting medium. First, a trial experiment was performed to determine the most effective dose of AsA application for maize plants to adopt in the main experiments. A single variety of maize, Golden, was grown in sand under pot conditions. Hoagland's nutrient solution was applied every week and four doses of AsA (0, 0.5, 1, 1.5, and 2 mM) were applied in root medium of maize seedlings every three days for two weeks. Then, in the main experiment conducted at the Botanical Garden at the University of Agriculture Faisalabad, two cultivars of maize i.e., Golden and Sadaf, were grown in the soil inside a wire house. The selected optimal dosage of vitamin C from trial experiment (1 mM) was applied to roots of maize plants for five weeks after every three days at seedling stage. Growth and physiological parameters were measured and statistically analyzed with the help of COSTAT software using analysis of variance (ANOVA) technique. When compared with the control plants, all plant parameters (root length, shoot length, leaf area, fresh and dry biomass, chlorophyll a and b content, soluble carbohydrates, osmotic potential, Potassium, Calcium, and Phosphorus content) were observed to be enhanced with AsA treatment. On the other hand, application of ascorbic acid decreased the sodium content of both roots and shoots of the treated plants. Moreover, genotype Sadaf demonstrated relatively better results in comparison to genotype Golden.

KEYWORDS: Maize, ascorbic acid (AsA), vitamin C, root application, growth, physiological, statistical analysis.

1 INTRODUCTION

Maize (corn, *Zea mays* L.) is an important species of the Poaceae family. Word *Zea* comes from Greek word meaning, "sustaining life" and *mays* is word of Taino language meaning "life giver". It is the third most important cereal crop in Pakistan, after wheat (*Triticum aestivum*) and rice (*Oryza sativa*). It has annual production of ~3.037 million tons with an average grain yield of 2,864 kilograms per hectare (Tariq and Iqbal, 2010), of which ~97% is produced in Punjab and KPK (formally NWFP) provinces of Pakistan. KPK alone accounts for ~57% of total cultivation area and ~68% of the total yield (Khan *et al.*, 2016).

Maize is a very healthy food with significant nutritional value containing ~72% starch, ~10% protein, ~8% fibre, ~4% oil, ~3% sugar, and ~1.72% ash (Chaudhary, 1983). It is chief food source for millions of people and is integral part of global food security (Campos *et al.*, 2004). It is a great source of vitamins A, B, and E and other important minerals. Research shows that a diet enriched in maize may lower the risk of high blood pressure and prevent from neural tube defects at birth (*Bushra et al.*, 2019). Maize grains are greatly used to produce cornflakes, cornstarch, grain cake, corn oil, dextrose, gluten, lactic acid, and acetone, which are used in several industries including textile, fermentation, foundry, and food industries (Mahama, 2021). It is also used to produce polymers, antibiotics, and ethanol.

Water, salt, and temperature stresses are the three main abiotic factors most likely to slow or hinder crop growth and healthy development resulting into nutritional imbalance, reduced water uptake and crop yield (Tommasi *et al.*, 2001). The increasing severity of pollution, soil erosion and degradation, and water scarcity are all steadily contributing towards the increase in the abiotic stresses (Suman and Kalpana, 2013).

Ascorbic acid or vitamin C acting as an enzyme cofactor is one of the greatest scavengers to shield plants from environmental stresses (Amin *et al.*, 2009). It helps in electron transport reactions in the cell membrane or plastids (Noctor and Foyer, 1998). Ascorbate (anionic form of AsA) is an effective antioxidant and is known due to its inimitable functions as plants suffer less from reactive oxygen species in

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the presence of ascorbate (Asada and Takahashi, 1987). Ascorbate elongates the root system of plants (Xuewen *et al.*, 2018and helps plants to better withstand periods of drought stress (Darvishan *et al.*, 2013). Treatment of maize with 0.5mM salicylic acid, 0.5mM ascorbic acid, and 0.1% Tween-20 relieved the detrimental effects of drought stress and improved cob diameter, biological yield, and grain production (Qasim *et al.*, 2019).

Different physiological parameters of plants comprising index of seed resistance, germination ratio, size of hypocotyl and radicle and water content were noted to increase by applying AsA (Reishi and Hassan, 2013). Khadr *et al.*, (2021) found that applying 200 mg/L of vitamin C to wheat seedlings helped reduce the negative effects of water deprivation stress on the plants' physical, morphological, and chemical characteristics. In response to Lead (Pb) stress, plants that were given ascorbic acid exogenously, had higher levels of relative water content (RWC), Rubisco activity, ATP sulfurylase (ATP-S), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg), and less chlorophyll degradation (Alamri *et al.*, 2018). Exogenously applied AsA induced enhanced osmo-protection and regulation of antioxidant defense system (Farooq *et al.*, 2020). Ascorbic acid also plays an important role in controlling the cell division and signal transduction (Pignocch *et al.*, 2003; Kerk *et al.*, 1995).

Ascorbic acid can be applied on plants exogenously in many ways, such as by spraying the leaves, pre-soaked treatment or in root medium. Based on the literature study, most of the researcher has investigated the effects of exogenously applied AsA under stressed conditions on maize plants. However, in Pakistan, it is usually grown in areas with abundant fresh water and normal soil conditions. Therefore, this study intends to examine the effectiveness of AsA application in rooting medium as a growth regulator to enhance the health and productivity of maize plants under normal cultivated conditions. The main objectives of this study are (i) to find the optimal dosage of AsA that can enhance the yield of maize under normal conditions, (ii) to quantify the effectiveness of different concentrations of AsA on different physiological and growth parameters on two maize cultivars i.e., Sadaf and Golden. The result of this study would benefit to understand the plant behavior in terms of various growth parameters and to optimize the dosage for maximizing the crop yield under normal conditions.

2 MATERIALS AND METHODS

First, an optimization experiment was performed to find the most effective dose of Ascorbic acid (AsA) application for maize plants. A single variety of maize, Golden, was sown in sand under pot conditions. Hoagland's nutrient solution was applied every week and four doses of ascorbic acid (0.5, 1, 1.5 and 2 mM) were tested by applying to the roots of the maize seedlings after every 3 days for 2 weeks. The solution was made by mixing ascorbic acid in distilled water along with Tween-20 as a surfactant. The level at which maximum growth occurred was selected to continue in the main experiments to investigate the influence of applying ascorbic acid on two varieties of maize i.e., Golden and Sadaf. Seeds of maize were sown in plastic pots containing 10 kg soil. Total 12 pots were taken, 6 for each variety (3 for control and 3 for treatment with ascorbic acid). Each pot had 6 plants. Ascorbic acid treatment was started at 3 leaf (seedling) stage of growth and was applied after every 3 days for 5 weeks. The growth and physiological parameters described in Sections 2.1 and 2.2 were recorded after 5 weeks treatment of ascorbic acid.

2.1 GROWTH PARAMETERS

The plants were harvested five weeks after ascorbic acid was applied to the rooting medium. A meter rod was used for root and shoot length measurements (in centimeters). The fresh weight (in grams) of plants was calculated using an electronic/digital balance. Dry weight (in grams) was recorded after samples were dried in an oven until their weight became constant. Area of the leaves (in square centimeters), was determined by using the following formula (El-Sahookie, 1985):

Leaf Area
$$(cm^2)$$
 = Leaf Length × Leaf Width × 0.75

2.2 PHYSIOLOGICAL PARAMETERS

Chlorophylls a, b and carotenoids were extracted, and their concentration was determined from fresh leaf samples following Arnon (1949):

$$\begin{split} \mathit{Chl.\,a}\;(mg\;ml^{-1}) &= [12.7\;(OD\;663) - 2.69\;(OD\;645)] \times \frac{V}{1000} \times W \\ \mathit{Chl.\,b}\;(mg\;ml^{-1}) &= [22.9\;(OD\;645) - 4.68\;(OD\;663)] \times \frac{V}{1000} \times W \\ \\ \mathit{Carotenoids}\;(g\;ml^{-1}) &= \frac{Acar}{Em^{100\%}} \times 100 \end{split}$$

$$Acar = [(OD\ 480) + 0.114\ (OD\ 663) - 0.638\ (OD\ 645)]$$

$$Em^{100\%}Cm = 2500$$

Where OD, V and W are optical density at certain wavelength (e.g., 480, 645 and 663 nm), the extracted volume of leaves (ml) and the fresh leaves tissue weight (g), respectively.

Calculations of total soluble carbohydrates were performed using the method of Hedge and Hofreiter (1962). Samples of leaves were frozen at -20°C for seven days. After the frozen leaves were thawed, and the cell sap was taken out. An osmometer (VAPRO) was used to find Osmotic potential of the sap. A flame photometer was used to measure the concentration of Sodium, Potassium, and Calcium ions, while a spectrophotometer (Hitachi-U2001, Tokyo, Japan) was used to measure the concentration of Phosphate ions.

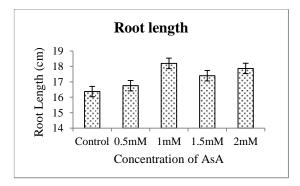
2.3 STATISTICAL ANALYSIS

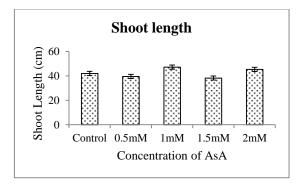
Statistical analysis of the data was determined using the technique of analysis of variance (ANOVA) in COSTAT software. Least Significant Difference (LSD) was calculated to get the differences among the means (Steele *et al.*, 1997).

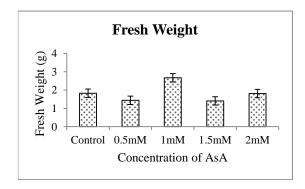
3 RESULTS AND DISCUSSION

3.1 INITIAL OPTIMIZATION EXPERIMENT

When different concentrations of vitamin C (ascorbic acid) (0, 0.5, 1, 1.5 and 2 mM) were applied to the maize plants, a noticeable increase was detected in all growth parameters including shoot length (cm), root length (cm), fresh weight (g) and dry weight (g). The most prominent increase was observed at the level of 1mM application of ascorbic acid (Figure 1). This dosage was selected as optimal within the current scope of study for main experiments.







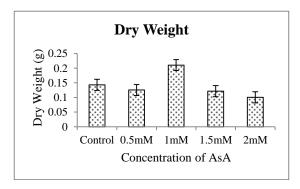


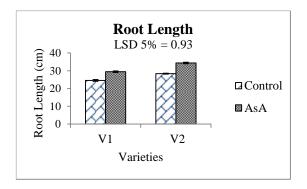
Fig. 1. Comparative impact of different concentrations of ascorbic acid (0.5mM, 1mM, 1.5mM and 2mM) by applying in rooting medium on various growth parameters

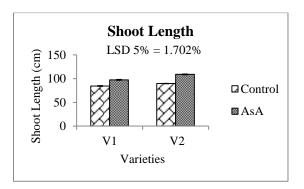
3.2 MAIN EXPERIMENT

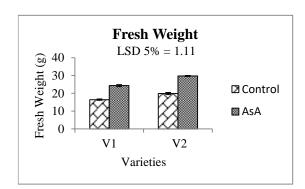
A deeper and longer root system is considered advantageous for crop survival under severe environmental conditions (Conklin, 2001). For the same reasons, having longer shoots results in a healthier plant growth, particularly during the vegetative stage when the plant can absorb more light and produce more simple sugars (Bakry *et al.*, 2013). In the present study, an increase was detected in both root and shoot lengths indicating ascorbic acid as a better growth regulator when applied in rooting medium in 1 mM concentration.

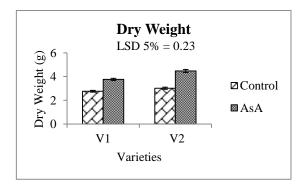
(Figure 2; Table 1). Larger plant roots and stems may be facilitated by vitamin C's association with increased meristematic cell division (Liso *et al.*, 1998). Several researchers have reported the similar results including Fazlali *et al.* (2013), Razaji *et al.* (2012), Khan *et al.* (2010), and Farhat *et al.* (2011) who noted the similar effect of ascorbic acid in terms of root and shoot growth on pumpkin, safflower, broccoli, and *Monstera delicious*, respectively.

Enhancement in fresh and dry biomass directs healthier look of maize plants (Behairy *et al.*, 2012). Both varieties of maize demonstrated an increased biomass in this experimental study, but Sadaf showed slightly higher growth (Figure 2; Table 1). Khan *et al.* (2006) and Hassan *et al.* (2021) also reported that fresh and dry weight of root and shoot was improved by action of ascorbic acid in wheat and *Hordeum vulgare* L, respectively. Similarly, higher photosynthesis potential is directly related to larger leaf area (Wright *et al.*, 2006). In this research, vitamin C application to the roots enhanced the leaf area of treated plants than those in the control group for both varieties. Ejaz *et al.* (2012) and El-Quesni *et al.* (2009) also reported similar findings in their experiments on *Saccharum spp.* and *Hibiscus rosa sinuses* L, respectively.









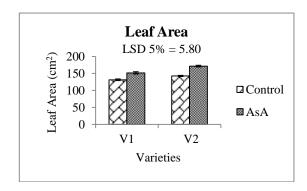
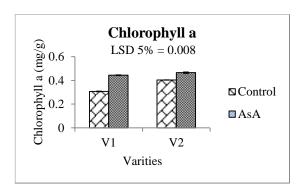


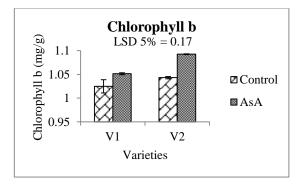
Fig. 2. Results of exogenously applied 1 Mm ascorbic acid via rooting medium on various growth parameters under normal cultivated conditions (Note: V1 and V2 are Golden and Sadaf verities, respectively)

df Source **Root Length Shoot Length** Fresh Weight **Dry Weight Leaf Area** Main effects 56.7675**^{*} 59.541075*** 210.0033333*** 0.684083** 732.3406273*** Genotype 1 89.1075*** 774.4133333*** 236.47440*** 4.5510083*** 1810.8188*** **AsA** 1 Interaction Genotype × AsA 1 0.800833333* 32.01333333** 2.970075 ns 0.151875 ns 2.8078137 ns

Table 1. Mean squares by ANOVA for various growth parameters under normal cultivated conditions for both genotypes

Note: ns = non-significant, *, **, *** = Values at 5%, 1% and 0.1% significant levels, respectively.





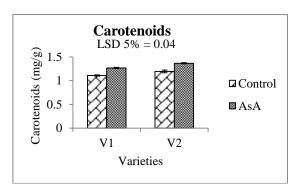


Fig. 3. Results of exogenously applied 1 Mm ascorbic acid via rooting medium on Chloroplast pigments and Carotenoids (Note: V1 and V2 are Golden and Sadaf verities, respectively)

The green pigments chlorophyll a and b found in chloroplasts are important for photochemical processes and rate of photosynthesis (Tappel, 1977; Taiz and Zieger, 2006). An increase in photosynthetic rate leads to the increased plant growth. In this investigation, with the application of AsA, both Sadaf and Golden varieties developed increased chlorophyll a, b, and carotenoid contents, with the former showing slightly better results (Figure 3; Table 2). An increase in photosynthetic pigments might be due to the fact that as an antioxidant, ascorbic acid has the capability to alleviate the damaging impacts of stress on plants by nullifying damaging oxidants that are reported to harm membranes of cell such as the thylakoid membranes in chloroplasts (Dolatabadian *et al.*, 2009). These results are in agreement with studies conducted by other investigators e.g., Tuna *et al.*, (2013) in maize, Dolatabadian and Jouneghani (2009) in wheat, Gaafar *et al.*, (2020) in the bean plants and Hassan *et al.* (2021) in *Hordeum vulgare* L., who also found that application of ascorbic increased the photosynthetic efficiency of plants in comparison with non-treated ones.

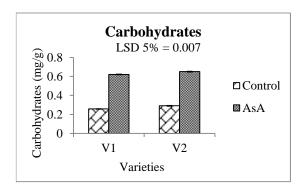
Table 2. Mean squares by ANOVA for chlorophyll a and b, carotenoids, osmotic potential, and soluble carbohydrates for both genotypes

Source	Df	Chl a	Chl b	Carotenoids	Osmotic Potential	Soluble Carbohydrates		
Main effects								
Genotype	1	0.10356 ***	0.0026572**	0.0156288**	0.0017256**	0.0029687***		
AsA	1	0.029512***	0.00431824***	0.103039***	0.0821182***	0.3924527***		
Interaction								
Genotype × AsA	1	0.004295***	3.9401e ⁻⁴ ns	0.0019757 ns	3.0984e ⁻⁴ ns	1.0089e⁻⁵ ns		

Note: ns = non-significant, *, **, *** = Values at 5%, 1% and 0.1% significant levels, respectively.

Plants get most of their energy from carbohydrates made during photosynthesis. Plants store them in the form of starch for later use (Stephen G. Pallardy, 2008). In the form of polysaacharides, carbohydrates take part in the construction of cellular structure, particularly cellulose makes a solid wall around the plant cell which provide the framework enabeling the plant to stand and extend. A notable increase was detected in carbohydrates content with vitamin C application in the present study in both varities of maize under normal conditions (Figure 4; Table 2). Farhat *et al.*, (2017) and Sadak *et al.*, (2010) also detected a rise in the carbohydrate contents of *Monstera delicious* and faba bean in their experiments.

Osmotic regulation is needed for plant cells to stay alive. Turgidity of cell is required for existence and appropriate working of plant metabolism (Taiz and Zeiger, 2006) as all the main reactions in plants occur with water availability (Khanna-Chopra & Selote, 2007). Osmotic potential keeps the plant cell turgid. Present study indicated a considerable increase in osmotic potential by applying ascorbic acid in root medium (Figure 4; Table 2). Farouk *et al.* (2011) also demonstrated in their research that under salinity, the osmotic potential of wheat increased by the application of ascorbic acid at 100 mg/L.



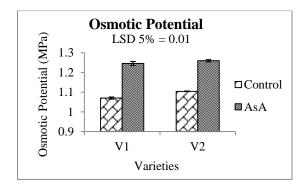
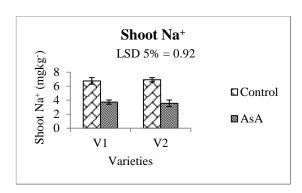


Fig. 4. Results of exogenously applied 1 Mm ascorbic acid via rooting medium on Carbohydrates content and Osmotic potential of both varieties (Note: V1 and V2 are Golden and Sadaf verities, respectively)



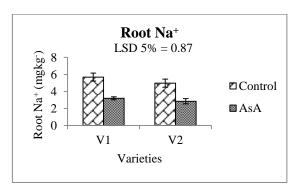


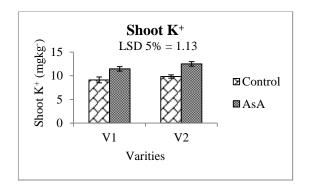
Fig. 5. Results of exogenously applied 1 Mm ascorbic acid via rooting medium on Root and shoot sodium (Na⁺) content of both genotypes (Note: V1 and V2 are Golden and Sadaf verities, respectively)

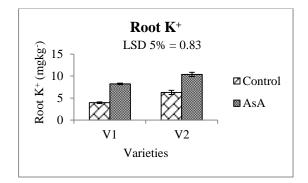
Table 3. Mean squares by ANOVA for Sodium (Na+) and Potassium (K+) contents in root and shoot of both genotypes of maize

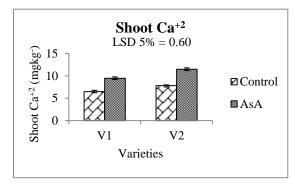
Source	df	Root Na⁺	Shoot Na ⁺	Root K⁺	Shoot K ⁺		
Main effects							
Genotype	1	0.852267 ns	0 ns	15.047201***	2.4075520 ns		
AsA	1	16.00368***	30.681612***	53.182826***	18.875208***		
Interaction							
Genotype × AsA	1	0.0947 ns	0.0946963 ns	0.0240755 ns	0 096302 ns		

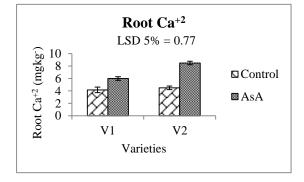
Note: ns = non-significant, *, **, *** = Values at 5%, 1% and 0.1% significant levels, respectively.

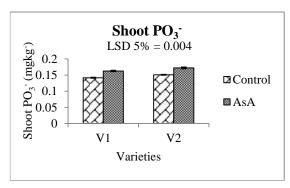
Large quantity of soluble salts in plant tissues disturbs the normal growth and function of vital crops, particularly in glycophytes grown under salt stressed habitat. Exogeneously applied ascorbic acid has positive effect against salt stress in improvement of plant growth (Hussein and Alva, 2014; Barus *et al.*, 2015). In this study a decrease in sodium (Na) content was observed especially in variety Golden (Figure 5; Table 3). Munir *et al.*, (2013) also supported this role of ascorbic acid as they studied AsA effect on callus culture of sugarcane grown under salinity. Aliniaeifrad *et al.*, (2016) also proved that ascorbic acid reduced the concentration of Na⁺ in olive plants.











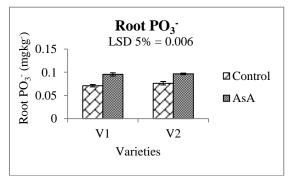


Fig. 6. Results of exogenously applied 1 Mm ascorbic acid via rooting medium on Potassium, Calcium and Phosphate contents of root and shoot (Note: V1 and V2 are Golden and Sadaf verities, respectively)

Plants need Calcium (Ca) to make strong cell walls and cell membranes. While deficiency of Ca leads to breakdown of cell walls and membranes and susceptibility to different diseases (Robert Norton, 2013). Potassium (K) is abundantly found cation and growth regulator in plants (White and Karley, 2010). It is an activator of numerous vital reactions like protein synthesis, transport of sugar, metabolism of Ca and N and photosynthesis. It is also a key factor in yield and quality of plant (Marschner, 2012; Oosterhuis *et al.*, 2014). Phosphorous (P) plays vital role in construction and expansion of root system, ultimately in crop yields (Bén *et al.*, 2015; Sun *et al.*, 2016). In present investigation, an increase was detected in Ca, P and K content of both cultivars especially in variety Sadaf (Figure 6; Tables 3 and 4). This result is analogous to *Farhat et al.*, (2017), who indicated that foliar spray of vitamin C increased Phosphorus (P) and Potassium (K) content of *Monstera delicious*. Elwan *et al.*, (2007) also presented in their studies that application of AsA improved the Potassium and Calcium content of treated eggplant leaves as compared to non-treated ones. The concentration of K⁺ was reported to increase by AsA application on olive leaves as well (Aliniaeifrad *et al.*, 2016).

Table 4. Mean squares by ANOVA for Calcium (Ca+2) and Phosphate (PO3-1) contents in root and shoot of both genotypes of maize

Source	Df	Root Ca ⁺²	Shoot Ca ⁺²	Root PO ₃ -1	Shoot PO₃-1		
Main effects							
Genotype	1	6.0087976**	8.316675***	2.9994e⁻⁵ns	2.5846e ⁻⁴ **		
AsA	1	25.469817***	33.2667***	10.001496***	0.00113374***		
Interaction							
Genotype × AsA	1	3.513705*	0 ns	1.3764e-5ns	3.1212e ⁻⁸ ns		

Note: ns = non-significant, *, **, *** = Values at 5%, 1% and 0.1% significant levels, respectively.

4 CONCLUSION

This study investigated the effectiveness of ascorbic acid (AsA) as a growth regulator by applying in the root medium to enhance the health and productivity of maize under normal conditions. All the growth and physiological attributes including root and shoot length, leaf area, plant fresh and dry biomass, chlorophyll pigments, soluble carbohydrates, Osmotic potential and mineral (Potassium, Calcium and Phosphate) contents of root and shoot showed significant increase. On the other hand, the Sodium content of both root and shoot was lessened by application of AsA in both genotypes of maize (i.e., Golden and Sadaf). Genotype Sadaf showed better results compared to Golden. Based on these findings, ascorbic acid can be exogeniously applied to increase the growth and productivity of maize plants in Pakistan under normal conditions. Also, genotype Sadaf can be utilized to achieve better results with ascorbic acid application in root medium.

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