International Journal of Innovation and Applied Studies
ISSN 2028-9324 Vol. 47 No. 2 Dec. 2025, pp. 138-146

http://www.ijias.issr-journals.org/

Effect of Exogenously Applied Vitamin C (Ascorbic Acid) as Foliar Spray on the Growth of Maize (*Zea mays* L.)

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ABSTRACT: Ascorbic acid (Vitamin C) is a growth regulator that stimulates plant growth and development. First, a preliminary experiment was performed to find the optimal Vitamin C dosage for maize plants. A single genotype of maize, Golden, was grown in sand under pot conditions. Hoagland's nutrient solution was applied for seven days. Four doses of Vitamin C (i.e., 0.5, 1, 1.5and 2 mM) were applied as foliar spray on maize seedlings for two weeks. Then, in the main experiment, two genotypes of maize (*Zea mays* L.) namely Golden and Agaiti 2000 were grown in soil in a wire house at the Botanical Garden, the University of Agriculture in Faisalabad (Pakistan). Based on the preliminary results, the optimal dose (2 mM) of ascorbic acid was applied exogenously as foliar spray on leaves of both genotypes every third day for five weeks at seedling stage (3 leaf stage). Findings were noted both qualitatively and quantitatively in terms of different physiological and growth parameters. In addition, COSTAT software was employed to perform statistical analysis of quantitative readings for all parameters using analysis of variance (ANOVA) technique. It was observed that treated plants showed improved health and growth compared to non-treated plants with higher contents of physiological variables including chlorophyll, carotenoids, soluble carbohydrates, and minerals etc. and increased leaves, roots and shoots sizes and biomass. Moreover, Agaiti 2000 genotype was observed to show better results than genotype golden.

KEYWORDS: Maize, ascorbic acid (AsA), vitamin C, foliar spray, growth, statistical analysis.

1 INTRODUCTION

Maize is one of the most popular oldest and staple cereals crops. It is popular for its use as food, fodder as well as for medical purpose in the world. It is one of the highest yielding crops in Pakistan and considered as the third chief cereal crop after wheat and rice (AARI, 2022). Due to its expanded industrial consumption, maize crop is also called "the other gold". In Pakistan, Punjab and KPK are the two major provinces for maize production. Both provinces produce almost 97% of the total maize crop in country. At commercial level, grains of maize are considered significant to produce oil, starch, and glucose (Niaz and Dawar, 2009).

Maize food products such as cooked corn, flour bread, popcorn, and cornflakes etc. are beneficial for human body. They are high in nutrients, quickly break down into intermediate carbohydrates and dextrin, and thus, are easy to digest. The dextrose produced during the process has medicinal benefits and helps with constipation. Maize contains several minerals like magnesium, potassium, phosphorus, calcium, zinc and iron etc. which helps to keep bones and kidney healthy. It also functions as an antioxidant to support our health (Jacinto *et al.,* 2018). A significant portion (~66%) of maize production is used as feed for animals/birds and for human consumption. Wet milling is second largest industry which consumes ~30% total maize yield and remaining 4% is generally used for seed and other applications (AARI, 2022).

Since plants cannot move, they must adapt to survive the severe environmental and natural conditions in their cultivation habitat. Because of environmental stresses such as drought or water stress, the crops can lose about two-thirds of their yield (Bray *et al.,* 2000; Manivannan*et al*., 2008).Salt or salinity stress is another such condition which adversely affects crops' agronomic and physiological characteristics and consequently, plants' yield (Gómez-Pando *et al.,* 2010).In response to these abiotic stresses, plants activate a wide range of enzymatic and non-enzymatic defense mechanisms to protect themselves from oxidative distresses generated by reactive oxygen species (ROS) (Ashraf, 2009). To minimize and control their adverse impacts, it is crucial to fully understand the biochemical, molecular, and physiological processes that stop plants from growing and developing normally (Razmjoo *et al*., 2008).

The effects of salt stress on plant growth and yield can be lessened by using exogenously applied growth regulators, non-enzymatic antioxidants, and fertilizers (Kaya *et al*., 2010). Vitamin C applications on plant's roots reduces salinity stress and raises quantity of cytosolic free Ca^{2+} ions (Makavitskaya *et al.,* 2018).It was reported that ascorbic acid applications raised nutrients (N, K^{+}), chlorophyll contents and all growth attributes under salt (NaCl) stress. (Alhasnawi *et al.,* 2015).Application of ascorbic acid also decreased microbial and antioxidant potential of plants under salinity stress and boosted their growth as well as the seed germination (Neelma *et al*., 2021; Chen *et al*., 2021).

Similarly, it has been demonstrated that exogenous application of ascorbic acid can reduce the detrimental consequences of water stress on plants (Khalil *et al*., 2010). Ascorbic acid (AsA /Vitamin C) is a powerful antioxidant that helps to detoxify the reactive oxygen bodies (Qian *et al*., 2014). Seed production, photosynthetic contents, carbonic anhydrase activity, general health, and antioxidant production are all negatively impacted by water stress. Ascorbic acid application helped to mitigate these negative impacts under water stress (Alaa A. Gaafar *et al*., 2020).

As literature survey shows, plants face adverse environmental conditions throughout their life cycle. Although, Vitamin C or ascorbic acid has been tested against salt/drought stress in various plant species, significance of this vitamin has not been thoroughly explored under normal conditions. In Pakistan, maize is usually grown in areas with abundant fresh water and normal soil conditions. Investigating the effects of Vitamin C application on maize plant would benefit to understand the plant behavior /response in terms of various growth parameters and to optimize the dosage for maximizing the crop yield under normal conditions. To address this gap, present study was designed to analyse the effectiveness of using ascorbic acid (AsA or Vitamin C) as a growth hormone to promote growth of maize plants and increase the productivity under normal conditions.

2 MATERIALS AND METHODS

First, to find the most effective dose of ascorbic acid (AsA) for maize plants, an optimization experiment was conducted. 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, or 2 mM) were applied by spraying maize seedlings after every 3 days for two weeks. Ascorbic acid and distilled water were mixed, and Tween-20 was added as a surfactant to create a foliar spray solution. The level at which maximum growth occurred was selected. This level (2 mM) was used to examine the effectiveness of AsA on two maize varieties. i.e., Golden and Agaiti 2000. Seeds of maize were sown in plastic pots containing 10 kg soil. The temperature and humidity were not controlled to closely represent the actual field conditions and they varied between 25-30^{o}C and 20-40%, respectively. Direct sunlight was used during the whole day. There were total of 12 pots, 6 for each variety (3 control and 3 treated with ascorbic acid). Each pot had 6 plants. Ascorbic acid treatment as foliar spray was started at 3 leaf (seedling) stage of growth and was applied after every 3 days. The growth and physiological parameters as described in following Sections 2.1 and 2.2 were recorded after 5 weeks treatment of ascorbic acid.

2.1 GROWTH PARAMETERS

The plants were grown and treated with ascorbic acid for 5 weeks before being harvested. A measuring tape was used to determine the root and shoot length in centimeter (cm). The fresh weight of the plant was calculated using the top/electronic balance in grams (g). After the determination of fresh weight, the samples were dried in oven and then dry weight (g) was noted. Area of leaves (cm^2) was determined by using following formula (El-Sahookie, 1985):

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

2.2 PHYSIOLOGICAL PARAMETERS

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

$$Chl. a\ (mg\ ml^{-1}) = [12.7\ (OD\ 663) - 2.69\ (OD\ 645)] \times \frac{V}{1000} \times W$$

$$Chl. b\ (mg\ ml^{-1}) = [22.9\ (OD\ 645) - 4.68\ (OD\ 663)] \times \frac{V}{1000} \times W$$

$$Carotenoids\ (g\ ml^{-1}) = \frac{Acar}{Em^{100\%}} \times 100$$

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

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

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.

Determining of total soluble carbohydrate content was done using the Hedge and Hofreiter technique (1962). Leaf samples were frozen at -20^{0}C for a week followed by thawing to extract the cell sap. To determine the sap's osmotic potential an osmometer (VAPRO) was used. The concentrations of sodium, potassium, and calcium ions were measured using a flame photometer, while the concentrations of phosphate ions were measured using a spectrophotometer (Hitachi-U2001, Tokyo, Japan).

2.3 STATISTICAL ANALYSIS

To perform a statistical analysis, analysis of variance (ANOVA) technique was employed through COSTAT software. To determine the difference between the mean values, Least Significant Difference (LSD) was calculated (Steele *et al.,* 1997).

3 RESULTS AND DISCUSSION

3.1 OPTIMIZATION EXPERIMENT

The root length (cm), shoot length (cm), fresh biomass (g), and dry biomass (g) of maize plants were all increased after applying ascorbic acid as a foliar spray at 0, 0.5, 1, 1.5, and 2 mM concentrations. Ascorbic acid at a concentration of 2 mM showed the highest increase (Figure 1); hence this was chosen as the optimal dosage for the main experiment.

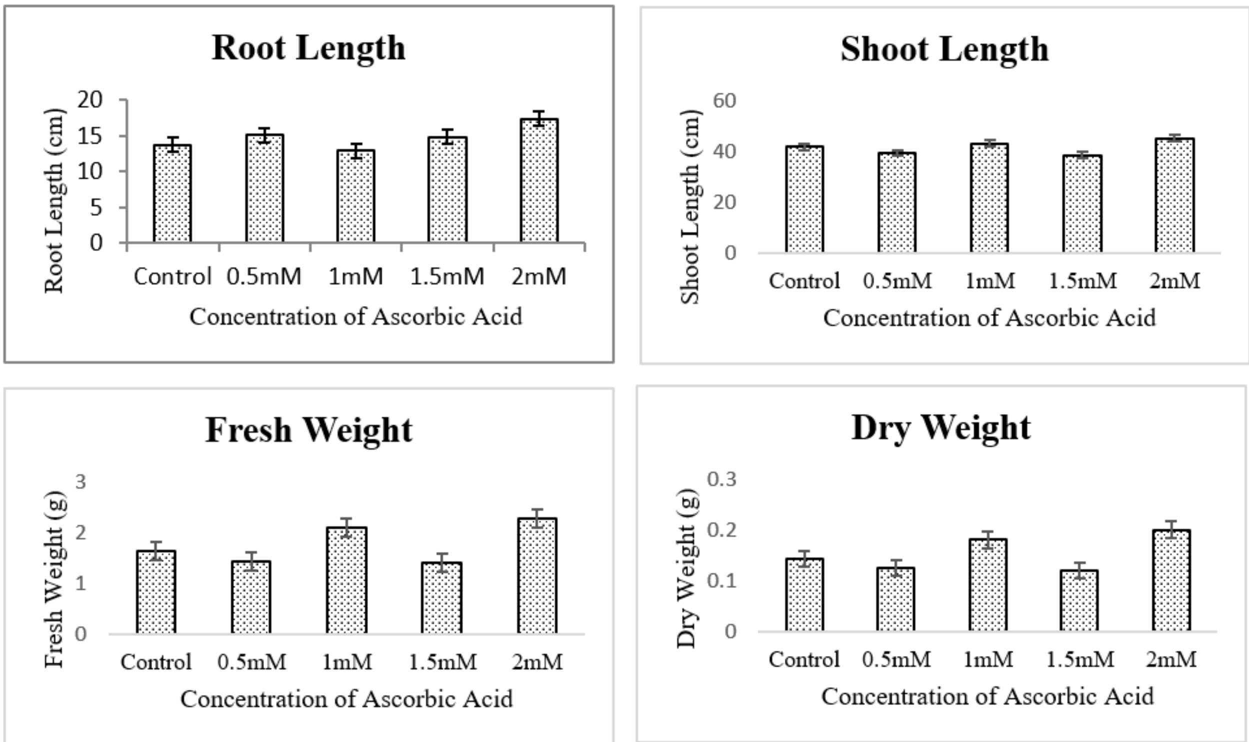

Fig. 1. Comparative effect of different concentrations of ascorbic acid (0.5mM, 1mM, 1.5mM and 2mM) as foliar spray on various growth parameters

3.2 Main Experiment

A plant with deep roots can absorb more water and nutrients than one with shallower roots. The chances of plants' survival in drought conditions are increased with stronger root system (Hoogenboom *et al*., 1987). Similarly, an expanded shoot system with larger leaves and longer shoots allow plants to absorb maximum light for increased photosynthesis and healthier growth (Bakry*etal.,* 2013). In this research foliar application of Vitamin C resulted in significantly longer roots and shoots in both maize cultivars. However, Agaiti 200 showed better results comparatively (Figure 2). This indicates that ascorbic acid has a positive influence on roots and shoots growth of treated plants in comparison to non-treated ones because it can regulate cell division, elongation, and differentiation of cells (Liso *et al.* (1988).These observations are in line with Fazlali *et al*., (2013), Ahmad *et al.* (2013), Razaji *et al*. (2012) and Hassan *et al.* (2021) who noted the same effects of Ascorbic acid on the root and shoot length growths for Pumpkin, spring Maize, safflower and *Hordeum vulgare* L. plants respectively.

Spraying ascorbic acid over the leaves of both genotypes also increased the fresh and dry weight of maize plants. The effect was found to be more pronounced in the Agaiti 2000 genotype. Khan *et al.* (2006) and Hassan *et al.* (2021) also supported this role of vitamin C in their respective studies. They reported that use of foliar spray of ascorbic acid improved the fresh and dry weight of the root and shoot in wheat and *Hordeum vulgare* L. In addition, ascorbic acid treatment also increased the leaf area of both maize varieties which is similar to the findings reported by Ejaz *et al*., (2012) for *Saccharum* spp. under the stress of salt. Larger leaf area can support more absorbance of light as well as increased number of stomata that leads to more photosynthesis and an enhanced plant growth (Weraduwage *et al*., 2015).

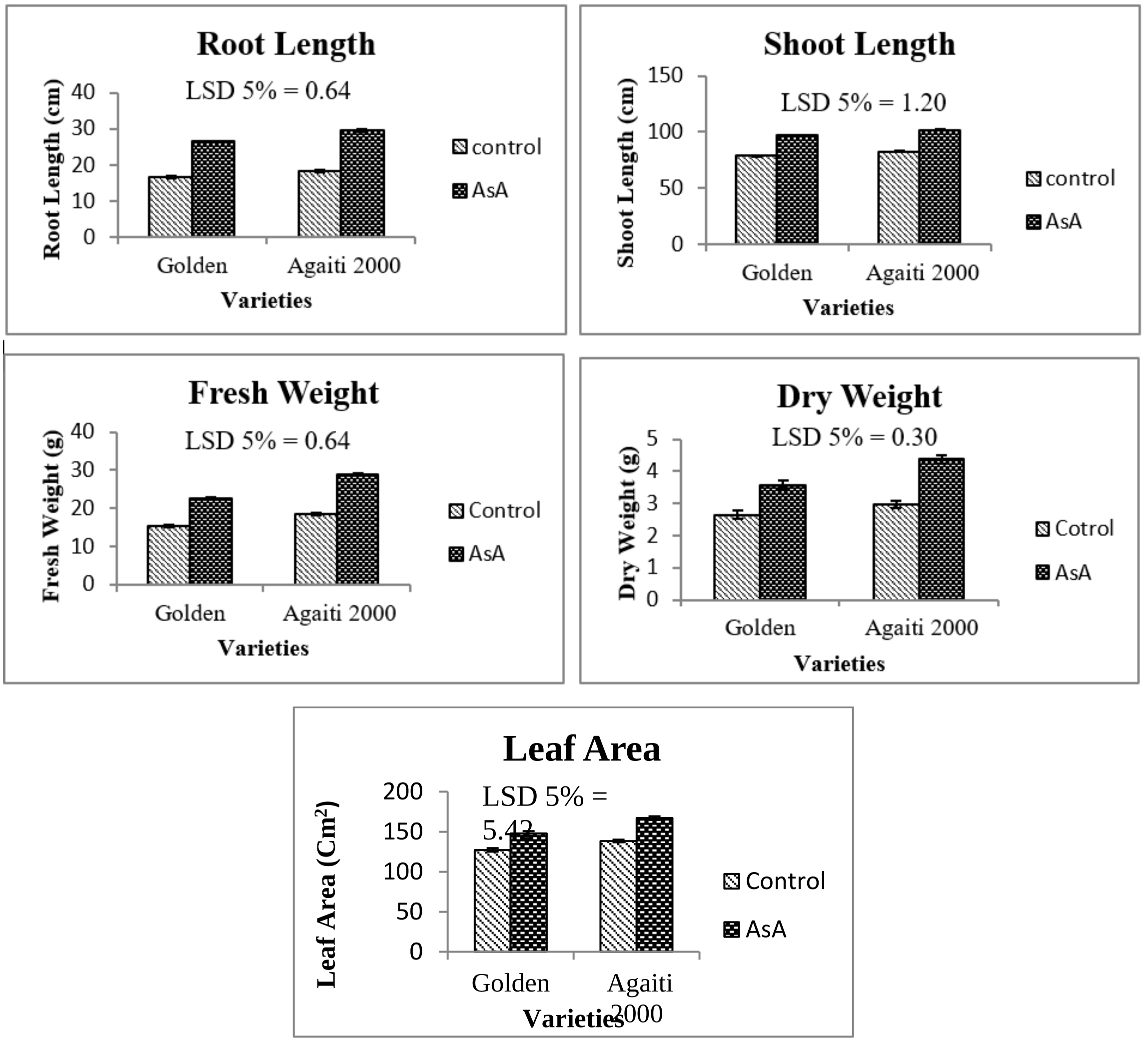

Fig. 2. Results of exogenously applied (foliar sprayed) 2 Mm ascorbic acid on various growth parameters under normal cultivated conditions

Chlorophyll a and b are green pigments of chloroplasts which have significant role in photochemical reaction and directly influence the rate of photosynthesis (Taiz and Zieger, 2006). Increased photosynthesis leads to rise in plant growth. In the present study, ascorbic acid application increased the contents of chloroplast pigments and carotenoids in both genotypes. However, genotype Agaiti 2000 showed slightly higher levels of chlorophyll a, b, and carotenoids than Golden (Figure 3). These findings are supported by studies conducted by other researchers e.g., Tuna *et al*., (2013) in maize, Dolatabadian and Jouneghani (2009) in wheat, and Hasan *et al*., (2021) in *Hordeum vulgare* L. who also found that foliar application of ascorbic acid increased the photosynthetic efficiency of plants in comparison to non-treated ones.

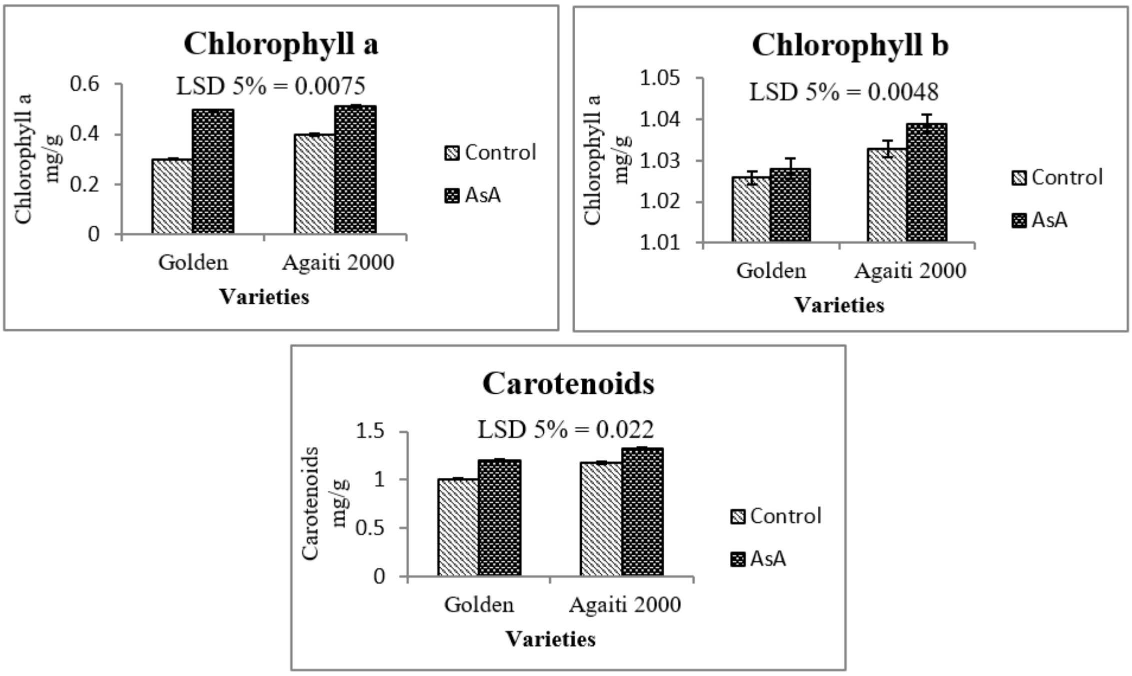

Fig. 3. Results of exogenously applied (foliar sprayed) 2 Mm ascorbic acid on Chloroplast pigments and Carotenoids

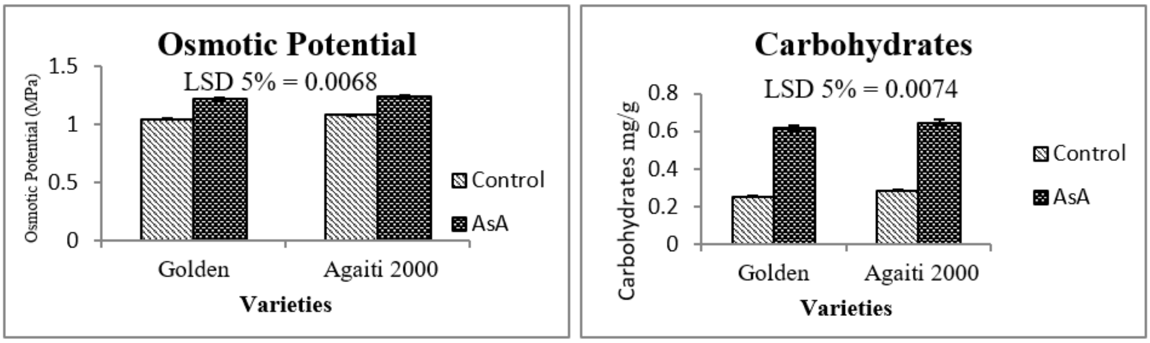

Fig. 4. Results of exogenously applied (foliar sprayed) 2 Mm ascorbic acid on Osmotic potential and soluble Carbohydrates

Carbohydrates formed through photosynthesis are the building blocks of many chemical compounds and are the key source of energy. Additionally, they have a pivot function as signaling molecules like phytohormones. They also play role in plant immunity (Smeekens *et al*., 2010). In the present study, ascorbic acid application increased total soluble carbohydrates of both genotypes (Figure 4). Sadak *et al*. (2010) also found a significant increase in total carbohydrates and total soluble sugars when ascorbic acid was applied on the shoots of faba bean plants under the stress of salt. On the other hand, osmotic regulation is important for plant cells to stay alive. It also facilitates leaf expansion and stomatal conductance (Westgate & Boyer, 1985). Moreover, osmotic potential of a cell keeps it turgid. In this study, when ascorbic acid was added as foliar spray, the osmotic potential was increased significantly. Farouk *et al*. (2011) also found that applying 100 mg/L of ascorbic acid increased the wheat's osmotic potential under salt stress.

Salt stress leads to decrease in seed germination (Zeinali*et al*., 2002). It also stimulates different plant metabolisms like ion toxicity, osmotic stress, and reactive oxygen species (Mittler, 2002). In present study, ascorbic acid (AsA) treatment decreased the amount of Na^+ in both roots and shoots. The effect was relatively more prominent in Golden genotype (Figure 5; Table 3). Alhakimi and Hamada (2001) also indicated that wheat grains soaked with AsA reduced the content of accumulated Na^+. Elwan*et al*., (2007) in eggplant and Elwan and El-Hamed (2012) in sweet Charlie plant also observed that AsA treatment mitigates the adverse effects of salinity by decreasing Na^+ content of plants.

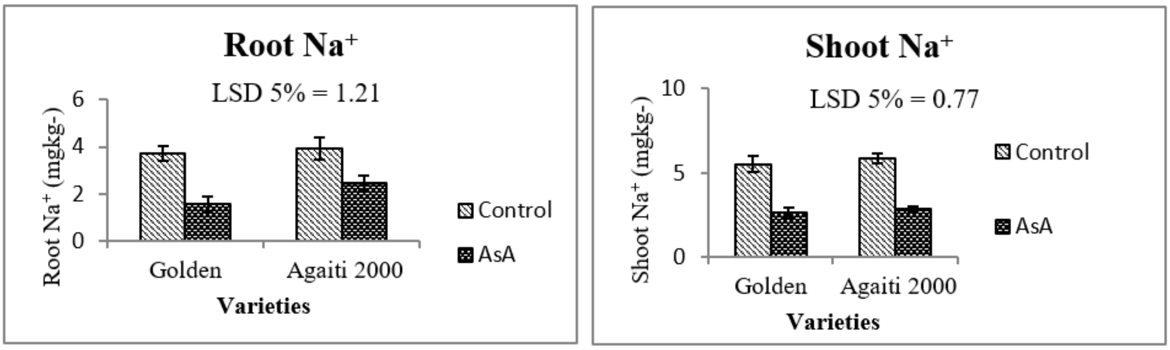

Fig. 5. Results of exogenously applied (foliar sprayed) 2 Mm ascorbic acid on Sodium (Na^+) content

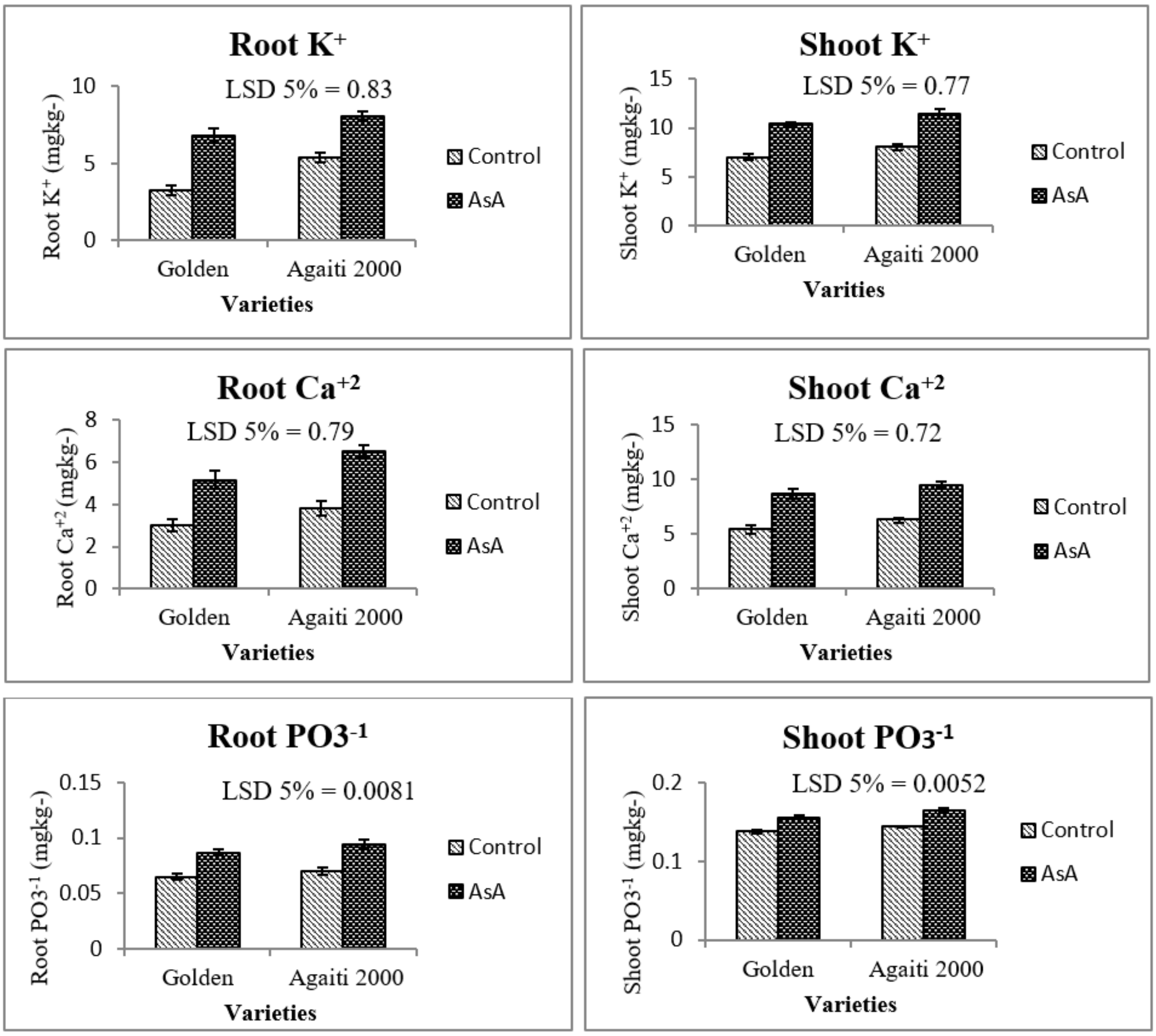

Fig. 6. Results of exogenously applied (foliar sprayed) 2 Mm ascorbic acid on Potassium, Calcium and Phosphate contents

Potassium as cation (K^+) is essential for plant growth (White and Karley, 2010). It acts as an activator of several vital enzymes. It also helps to improve photosynthesis, protein synthesis, transporting sugar, and metabolism of nitrogen and carbon. It has a vital role in plants' quality and yield improvement (Marschner, 2012; Oosterhuis*et al*., 2014). Calcium (Ca^{+2}) as a mineral is needed in construction of the cell wall, especially in middle lamella to hold the strands of pectin together (Eklund and Eliasson, 1990). It also takes part in cell division and plays a role of messenger in reactions to different environmental and hormonal signals (White and Broadley, 2003). Phosphate (PO_3^-) is another important mineral in DNA and RNA. It is needed for the formation of lipid bilayers in cell membranes. It is also needed for the reversible phosphorylation of proteins. In the cytoplasm of a typical plant, there are between 60 and 80µM of PO_3^- (Pratt *et al.*2009). To grow and develop normally, plants need more PO_3^- than the standard 10µM found in soil (Plaxton and Tran, 2011).

In present investigation, an improvement was examined in the Calcium (Ca^{+2}), Potassium (K^+) and Phosphorus (PO_3^-) content of both roots and shoots in both varieties of maize with the application of Vitamin C (Figure 6; Table 4). Elwan*et al*. (2007) showed that Vitamin C application increased the Potassium and Calcium content of eggplant leaves. Arab and Ehsanpour (2006) also observed that application of ascorbic acid to alfalfa plants under salt stress raised the level of acid phosphate under invitro conditions.

4 CONCLUSION

Overall, the foliar application of ascorbic acid under normal conditions and environment (general representation of most agricultural areas in Punjab and KPK) significantly increased all growth attributes and physiological attributes including root, shoot and leaves sizes, fresh and dry weight of plants, chloroplast pigments, osmotic potential, and mineral (Calcium, Phosphate and Potassium) contents of root and shoot. On the other hand, salt (Sodium) content in roots and shoots was decreased by application of ascorbic acid in both genotypes of maize (Golden andAgaiti2000). Genotype Agaiti 2000 showed slightly better results compared to the Golden. Thus, foliar application of ascorbic acid is effective in overall plant growth. Future extension studies can focus on exploring the effects of higher dosage application of vitamin C including additional diversity of genotypes.

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