The role of potassium and ascorbic acid on some growth and physiological responses in *Catharanthus roseus*

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Abstract

Different concentrations (1.5, 3.16, 15, and 30 mM) and forms (K_2SO_4 and KNO_3) of potassium were applied through Hoagland's nutrient solution. Ascorbic acid, 750 and 1500 mg L^{-1}, was sprayed twice on seedlings (age 68 and 78 days). Wet and dry weight, plant height, photosynthetic pigments, sodium, and potassium contents were measured at the end of the growing season. Also, total free amino acids was measured by HPLC. The excess of potassium increased the total wet (62%) and dry weight (54%), leaf area (31%), shoot and root height (49% and 15%, respectively), total chlorophyll (44%), and K^+/Na^+ ratio (100%) while the total free amino acids (2 times) and sodium content (28%) decreased. Ascorbic acid showed an almost similar trend under potassium in the mentioned traits, but it did not affect the root height and total sodium and potassium contents. There was a positive interaction between the potassium and ascorbic acid on the plant weight and height, leaf area, photosynthetic pigments, and K^+/Na^+ ratio. Regarding the positive effect of potassium and ascorbic acid on the growth parameters along with the similar changes on the photosynthetic pigments and the K^+/Na^+ ratio with the reduction of total free amino acids, it may be argued that both treatments improved plant growth through plant stability, increased photosynthetic rate, and production of more protein and other metabolites in *Catharanthus roseus*. Due to the importance of chemical compounds in *Catharanthus roseus*, any increase in the growth parameters leading to an increase in the yield of the plant can be very valuable.

Keywords: *Catharanthus roseus*; physiological parameters; K_2SO_4, KNO_3; ascorbic acid

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Introduction

Medicinal plants are important economically because they are a rich source of secondary valuable metabolites and compounds for medicines (Nagaveni et al., 2018). *Catharanthus roseus* (*C. roseus*) belongs to the Apocynaceae family and is an evergreen plant, perennial herbaceous subshrub with creeping stems and leathery and elliptic leaves that grow up to 100 cm in height. This plant is one of the most important medicinal plants for cancer therapy (Nisar et al., 2016). *C. roseus* produce useful chemical compounds such as flavonoids and alkaloids. Alkaloids are the most important compounds of *C. roseus* to treat diabetes, blood pressure, and cancer. These compounds are mostly made or accumulated in the leaves and

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stems (Das et al., 2017). Therefore, an increase in plant growth leads to more production of these valuable compounds.

The composition of plant nutrients is an important factor for growth and development (Nagaveni et al., 2018). Therefore, different concentrations of plant nutrients could change the quantity and quality of the medicinal components. Consequently, different levels of the essential macro-nutrients can affect the plant height, leaf area, and yield (Nagaveni et al., 2018). In *C. roseus*, the use of phosphate and sucrose as nutrients caused an increase in dry weight and the number of cells in cell suspension culture (MacCarthy et al., 1980). There are different reports that increasing the amount of potassium increased significantly the height and yield in *Zea mays* L. (Ortas, 2018) as well as the plant height, leaf area, plant growth, wet and dry weight, and biomass in pepper (Hussein et al., 2012).

Potassium (K^+) is one of the most essential macro-nutrients that affect biochemical and physiological processes, and it is necessary for optimum growth and metabolism in plants (Wang et al., 2013). External application of K^+ was shown to have a significant effect on cell elongation and growth in shoots and roots (Wang et al., 2013; Islam et al., 2004) and increase total dry weight of plant (Clavijo-Sánchez et al., 2015) and yield and quality (Hu et al., 2017; Guo et al., 2019). Furthermore, K^+ has an important effect on photosynthetic pigments (Arafa et al., 2011), enzyme activation, photosynthesis, phloem transport (Marschner, 2012), amino acid and protein metabolism (Hu et al., 2016), and cation-anion balance (Wakeel et al., 2011).

Ascorbic acid (AsA) acts as an important compound in the metabolic processes of plants (Noctor and Foyer, 1998). AsA has a remarkable role in physiology, growth, development (Khan et al., 2011), and normal function in plants (Alamri et al., 2018). AsA has several effects on cell division, cell wall expansion, and other developmental processes (Smirnoff, 1996; Asada, 1999; Venkatesh and Park, 2014). In addition, it has different effects on the photosynthetic pigments such as chlorophyll a and b, and photosynthesis rates in plants (Ameer et al., 2010; Naz et al., 2016; Bybordi, 2012). There are also different reports on the effect of AsA on amino acid and protein metabolism in different plants (Gebicki et al., 2010; Min et al., 2020).

It is well established that the amount of photosynthetic pigments are affected by the amount of K^+ in plants (Hussein and Alva, 2014; Hu et al., 2017; Shafeek et al., 2005). In addition, sufficient K^+ led to development of leaf area in plants (Zhao et al., 2001; Cheema et al., 2012). The optimum level of K^+ properly maintained the photosynthesis and thus increased the plant growth parameters (Wang et al., 2015) such as plant height, leaf area, and root length (Huda et al., 2010; Hussain et al., 2011; Kwizera et al., 2019). On the other hand, AsA increased the leaf area which contributed to the photosynthesis process of the plant (Tanaka et al., 2009). Anti-oxidative properties of AsA prevented the degradation of chlorophyll (Zonouri et al., 2014) which led to a positive effect on photosynthetic pigments in Canola (Bybordi, 2012). It was reported that foliar application of AsA significantly raised plant height (Hussein and Alva, 2014), leaf area (Ejaz et al., 2012), and the growth of Rosebushes (Herrera-Martínez et al., 2013) as well as the fresh and dry weights in sugarcane plants (Ejaz et al., 2012). In addition, some amino acids decreased under the exogenous application of AsA in spinach (Min et al., 2020). Amino acids are precursors for the biosynthesis of proteins in various plants (Bennett and Wallsgrove, 1994). Therefore, decreased free amino acids can be the result of further production of proteins in AsA-treated plants which leads to increasing the growth parameters. In addition, in cotton leaves (Hu et al., 2017), corn leaves (Hsiao et al., 1970), tobacco (Koch et al., 1974), and barley (Helal et al., 1979) free amino acid contents increased under K^+ deficiency. Since an increase in the free amino acid content means a decrease in the protein content, a reduction in protein content in K^+ deficiency leads to a reduction in growth parameters. Consequently, for a normal plant function, controlling K^+ concentration and K^+/Na^+ ratio is very important (Wang et al., 2013). External application of K^+ by adjusting the tissue ionic balance reduced the Na^+ uptake in peanut (Chakraborty et al., 2016). So, the exogenous K^+ decreases the uptake of harmful nutrients that leads to increased yield in plants (Ganie et al., 2017).

There is little information related to the interaction effect of K^+ and AsA on the growth and physiological responses in *C. roseus*. However, positive interaction was reported between the K^+ treatment and the foliar spraying of AsA in sweet potato and total yield in garlic (*Allium sativum* L.) (El-Morsy et al., 2010). The highest quantity of growth characteristics and the total yield were observed in the combination of K^+ and AsA in sweet potato (El-Seifi et al., 2014).

Due to the high price (Collin, 2001) and low amount of secondary metabolites like alkaloids in *C. roseus*, there is a great attraction to research for this plant (Moreno et al., 1995). Comparison between the amount and difficulty of alkaloids obtained by alternative methods including chemical semi-synthesis, cell and tissue culture, and biotechnological approaches (Verpoorte et al., 1997) indicated that *C. roseus* remains the only valuable source of these compounds (Verpoorte et al., 1999). Therefore, due to the value of alkaloid compounds and the role of plant production on the amount of these compounds, any improvement in the growth parameters of *C. roseus* can be very valuable.

Regarding the effect of K^+ and AsA on the growth and physiological parameters of different plants, it can be assumed that K^+, AsA, and their interaction may have an impact on the growth and physiological parameters in *C. roseus.* Therefore, this research was conducted to evaluate the effect of different concentrations of K^+ and/or AsA on plant height, leaf area, and weight as growth parameters, the amount of photosynthetic pigments as photosynthetic contributors, and total free amino acid content as physiological responses, and the amount of sodium as a harmful ion and potassium as a necessary ion for plant growth in *C. roseus*.

Materials and Methods

Plant materials

Seeds of *Catharanthus roseus* (L.) G. Don (*C. roseus*) were obtained from the Medicinal Plant Research Center, Isfahan, Iran. Sterilized seeds were germinated in Petri dishes using a water agar culture medium. Uniform 3-day-old germinated seeds were transferred to pots, filled with perlite and irrigated with half-strength Hoagland's nutrient solution. The seedlings were kept in a growth chamber at a temperature of 28 °C and 65% relative humidity under 16/8 h (light/dark) photoperiod at an irradiance of 450 µmol m^{-2} s^{-1}.

Growth curve

To determine the exact time of ascorbic acid treatment from 30-day age of the plant, every ten days, growth parameters like fresh weight were examined, and a growth curve was drawn.

Plant treatments

Potassium was added to half-strength Hoagland's nutrient solution in the range of 1.5 (deficient), 3.16 (optimum), 15 (excess), and 30 mM of K^+ with KNO_3 or K_2SO_4 forms. Ascorbic acid (AsA) was sprayed twice manually on the surface of leaves: first, when the plants were 68 days old, and second, at the age of 78 days (growth phase diminishing time). The concentration of AsA in the solution was 750 or 1500 mg L^{-1} (containing 0.1% Tween 20 as a surfactant agent). The spraying was done until dripping from the leaves. Plant samples were collected at the harvest time at the end of the vegetative growth season when plants were 90 days old (before the flowering phase).

Ascorbic acid measurement

Total AsA of the leaves in the control and treated plants were measured. For this purpose, 0.2 g fresh leaf was rubbed in a cold mortar on ice with 2 ml cold extraction buffer containing 5% metaphosphoric acid and 1 mM EDTA and centrifuged at 15,000 rpm and 4 °C for 10 minutes. Then, 200 µl sodium phosphate buffer (150 mM) pH 4 and 200 µl (10 mM) were added to 200 µl of the extract and were shaken to homogenize. The solution was kept at room temperature for 30 minutes. Then, 100 µl N-Ethylmaleimide (5%) was added and the mixture was shaken to homogenize and the solution was kept at room temperature for 10 minutes. Afterwards, 400 µl trichloroacetic acid (10%), 400 µl orthophosphoric acid (44%), 400 µl 2-2 dipyridyl (4%) were dissolved in 70% ethanol, and 200 µl 3% iron chloride were added.

This was followed by shaking the solution to homogenize before it was kept for 60 minutes in a water bath at 37 ℃. The absorbance was measured at 525 nm wavelength by spectrophotometer (Shimadzu, Japan). The amount of ascorbic acid was calculated using a standard curve.

Wet and dry weight measurement

The roots were cleaned carefully with running tap water. The wet weight of leaves, shoots, and roots of the plant were measured immediately and the dry weight of the different parts (leaves, shoot, and root) of the plant were recorded separately after the samples were oven-dried at a temperature of 70 ℃ for 72 hours.

Plant height and leaf area measurement

The plant shoot and root heights were measured. Also, leaf area was measured using a leaf area meter (Li 3100, USA).

Photosynthetic pigments measurement

Chlorophylls a, b, and carotenoid were measured by the Arnon method (1949). First, 0.1 g of the leaf wore up in the cold mortar, on ice, and dark in 10 ml acetone 80% to obtain a homogeneous solution. Then, it was centrifuged at 4000 rpm. The supernatant was used to measure photosynthetic pigments at 663, 645, and 470 nm wavelengths using a spectrophotometer (Shimadzu, Japan) for chlorophylls a, b, and carotenoid, respectively. The amount of photosynthetic pigments was obtained by the following formulas:

$$\text{Chla (mg g}^{-1}) = [12.7 (A_{663}) - 2.69 (A_{645})] \times V/1000 \times W$$

$$\text{Chlb (mg g}^{-1}) = [22.9 (A_{645}) - 4.68 (A_{663})] \times V/1000 \times W$$

$$\text{Total Chla + b (mg g}^{-1}) = [20.2 (A_{645}) + 8.02 (A_{663})] \times V/1000 \times W$$

$$\text{Carotenoid} = [1000 (A_{470}) - 1.8 (\text{Chla}) + 85.02 (\text{Chlb})] / 198$$

(Chla: chlorophyll a, Chlb: chlorophyll b, A_{645}: absorbance at a wavelength of 645 nm, A_{663}: absorbance at a wavelength of 645 nm, A_{470}: absorbance at a wavelength of 645 nm, V: final volume, W: fresh weight)

Total free amino acid measurement by HPLC

First, 2 g of *C. roseus* fresh frozen leaves was homogenized with 10 mL methanol containing 2% formaldehyde using an ultra-homogenizer (Heidolph, Silent crusher M, Germany) at 10000 rpm. After 2 hours of incubation, the samples were centrifuged at 13000 rpm for 10 min. Then, supernatants were separated and dried. Total free amino acids were back-extracted with diethyl ether. After drying, each sample was solubilized in 1 mL HPLC mobile phase for analysis. HPLC configuration was: Knauer pump 6.1L (Azura), Shimadzu prominence fluorescence detector RF-20A (wavelengths of analysis: emission 450 and excitation 430 nm), Rheodyne injector fitted with sample loop 50 µL, syringe 50 µl SGE (made in Australia), C18 reversed-phase column (150 × 4.6 mm, 5 m), and Clarity software. The sample injection volume was 50 µL at a 1.2 mL min^{-1} flow rate (Salmanizadeh and Sahi, 2020).

Sodium and potassium measurement

Here, 0.01 g dry weight of the aerial parts and roots of the plant were crushed and transferred to experimental tubes. Then, 10 ml sulfosalicylic acid 3% was added to each tube and the tubes were closed and incubated in the refrigerator for 48 hours. After that, the tubes were centrifuged at 4000 rpm, and the amount of sodium and potassium were measured by flame photometer (405G). K^+ and Na^+ standard solutions were prepared separately (with different concentrations), and the related standard curves were prepared. Then, the amounts of sodium and potassium of the plant samples were calculated.

Statistical Analysis

The experiments were performed as a factorial layout in a completely random design with 3 replicates. Statistical analysis was performed using SPSS 22. Duncan's multiple test

ranges was used for mean comparison and Excel 2016 was used to draw the necessary figures.

Results

Growth curve

Based on the growth curve, the diminishing growth phase happened on day 78. The flowering phase happened after 90 days (Fig. I).

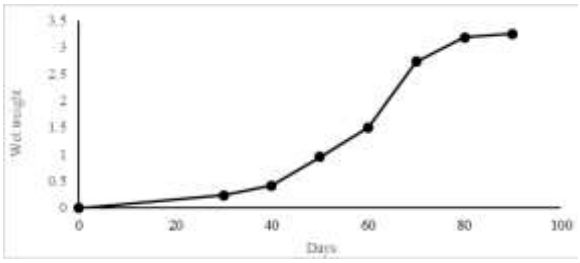

Fig. I. Growth curve of *C. roseus* plant

Effect of the spraying AsA on AsA content

The internal AsA in the treated plants showed higher AsA compared to the control plant. The data showed that different concentrations of exterior AsA had a significant effect on the content of internal AsA. The amount of AsA increased by about 20% or 33% in the leaves treated with 750 or 1500 mg L^{-1}AsA, respectively compared to the control plants (Fig. II).

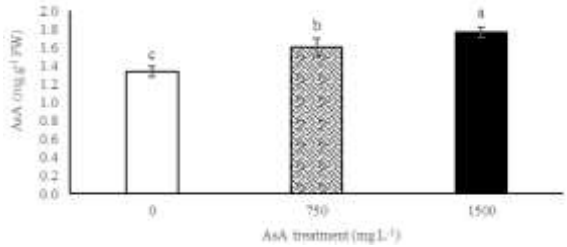

Fig. II. AsA content (mg g^{-1} FW) in the leaves of *C. roseus* plants; values are the means of three replicates and different letters indicate the significant differences among the mean values based on Duncan's multiple range test ($p \leq 0.05$). The bar lines represent the standard deviation (SD).

Effect of K^+ and AsA on wet and dry weight

Results demonstrated that there is a significant difference between K^+ deficient plants and control plants for the wet and dry weight (Fig. III). Therefore, wet weight almost decreased 48% in leaves, 45% in shoots, and 43% in roots under K^+ deficiency compared to control plants. In contrast, the excess of K^+ showed a significant rise in wet weight compared to control plants, and it increased 25% or 43% in the leaves, 75% or 130% in shoots, and 35% or 65% in roots under 15 or 30 mM K^+, respectively. In any case, the total wet weight decreased by 43% under K^+ deficiency while it increased by 36% under 15 mM and 62% under 30 mM K^+.

Dry weight in leaves, shoots, and roots decreased by almost 62%, 49%, and 45% under K^+ deficiency compared to control plants. In the excess of K^+ dry weight increased by 15% or 35% in leaves, 50% or 131% in shoots, and 30% or 70% in roots under 15 or 30 mM K^+, respectively compared to the control plants. In this case, total dry weight decreased by 58% but increased by 22% or 54% under 15 or 30 mM K^+, respectively compared to the control plants.

The data showed that ascorbic acid had a significant effect on wet and dry weights. Wet weight increased about 18% or 37% in leaves treated with 3.16 mM K^+ and 750 or 1500 mg L^{-1} AsA, respectively compared to the control plants (3.16 mM K^+ with no AsA). 750 or 1500 mg L^{-1}AsA had the same effect on the shoot and root, so increasing wet weight in the shoot and root was 30% in 750 or 1500 mg L^{-1}AsA compared to the control plants. Total wet weight increased about 21% and 35% under foliar application of 750 and 1500 mg L^{-1}AsA, respectively.

Interaction effect of 15 mM K^+ with 750 or 1500 mg L^{-1} AsA increased about 41% or 56% of the wet weight of leaves whereas 30 mM K^+ with 750 or 1500 mg L^{-1} AsA increased 63% compared to the control plants. In shoots and roots, 15 or 30 mM K^+ with two concentrations of AsA had the same effect. In this regard, the interaction effect of 15 mM K^+ and 750 or 1500 mg L^{-1} AsA resulted in an increase in shoot and root wet weight by 75% and 78%, respectively while 30 mM K^+ with 750 or 1500 mg L^{-1} AsA led to an increase by 82% and 130% in the wet weight of shoots and roots, respectively. Total wet weight increased about 56% and 66% in the plants treated with 15 mM K^+ and 750 and 1500 mg L^{-1} AsA while under 30 mM K^+ and 750 and 1500 mg L^{-1} AsA it increased by 72% and 79%, respectively.

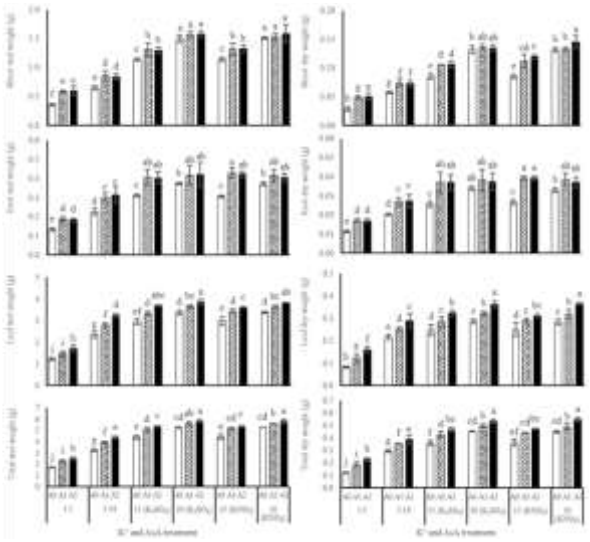

Fig. III. Dry and wet weight (g) in 90 day-old *C. roseus* plants; abbreviated letters A0, A1, and A2 are used for 0 (control), 750, and 1500 mg L^{-1} AsA, respectively. These concentrations were sprayed on leaf surfaces until dripping. Potassium (K^+) concentrations (mM) were used as either KNO_3 or K_2SO_4 in Hoagland's nutrient solution. Values are means of the three replicates and the different letters indicate the significant differences among the mean values based on Duncan's multiple range test ($p \leq 0.05$). Bar lines represent the standard deviation (SD).

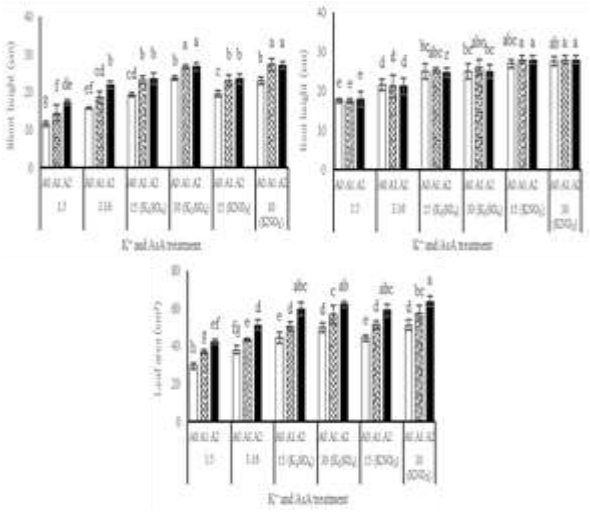

Fig. IV. Shoot and root height (cm) and leaf area (cm^2) in 90 day-old *C. roseus* plants; abbreviated letters A0, A1, and A2 are used for 0 (control), 750, and 1500 mg L^{-1} AsA, respectively. These concentrations were sprayed on leaf surfaces until dripping. Potassium (K^+) concentrations (mM) was used as either KNO_3 or K_2SO_4 in Hoagland's nutrient solution. Values are the means of the three replicates and different letters indicate the significant differences among the mean values based on Duncan's multiple range test ($p \leq 0.05$). The bar lines represent the standard deviation (SD).

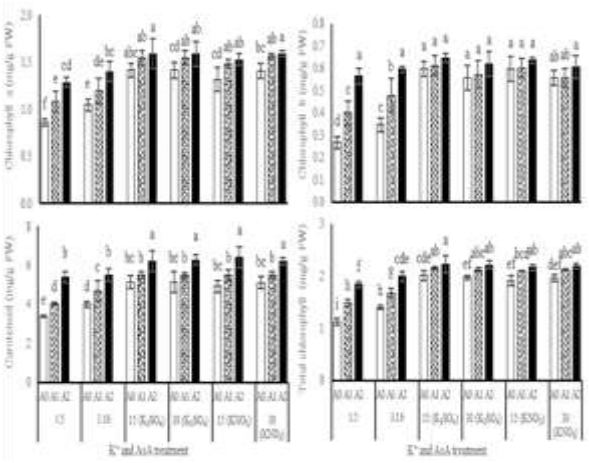

Fig. V. The amount of photosynthetic pigments (mg g^{-1} FW) in 90 days old *C. roseus* plants. ; abbreviated letters A0, A1, and A2 are used for 0 (control), 750, and 1500 mg L^{-1} AsA, respectively. These concentrations were sprayed on leaf surfaces until dripping. Potassium (K^+) concentrations (mM) was used as either KNO_3 or K_2SO_4 in Hoagland's nutrient solution. Values are the means of the three replicates and different letters indicate the significant differences among the mean values based on Duncan's multiple range test ($p \leq 0.05$). The bar lines represent the standard deviation (SD).

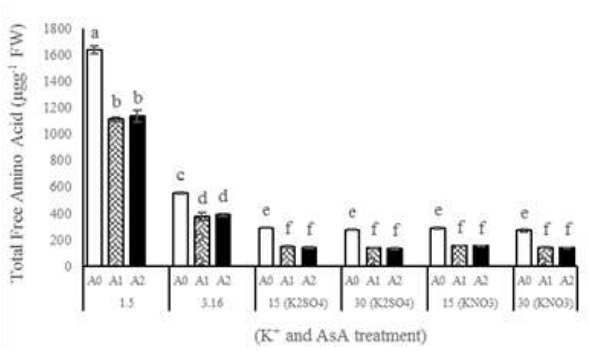

Fig. VI. Total free amino acids content (µgg^{-1} FW) in 90 days old *C. roseus* plants. ; abbreviated letters A0, A1, and A2 are used for 0 (control), 750, and 1500 mg L^{-1} AsA, respectively. These concentrations were sprayed on leaf surfaces until dripping. Potassium (K^+) concentrations (mM) was used as either KNO_3 or K_2SO_4 in Hoagland's nutrient solution. Values are the means of the three replicates and different letters indicate the significant differences among the mean values based on Duncan's multiple range test ($p \leq 0.05$). The bar lines represent the standard deviation (SD).

AsA, and the interaction effect of K^+ and AsA, dry weight changes had the same trend compared to the wet weight.

Effect of K^+ and AsA on shoot and root height

There was a significant difference between the effects of different concentrations of K^+ on the leaf area, root length, and shoot height (Fig. IV). K^+ deficient plants had a decrease about 22% in the leaf area, 36% in shoot height, and 18% in root length compared to control plants. In contrast, there were increases about 17% in leaf area, 22% in the shoot height, and 15% in root length in plants treated with 15 mM K^+ compared to the control plants. However, increases in leaf area and shoot height under 30 mM K^+ were about 31% and 49%, respectively. The differences between root height in 15 mM K^+ and 30 mM K^+ were no significant.

Different concentrations of AsA had different effects on the leaf area and 750 and 1500 mg L^{-1} AsA resulted in increases in leaf area about 14% and 34%, respectively as compared to the control plants (3.16 mM K^+ with no AsA). The effect of 750 and 1500 mg L^{-1} AsA on shoot height showed an increasing trend about 18% and 39%, respectively. AsA had no significant effect on root length.

In the combined effect of K^+ and AsA, data indicated that increases in leaf area under 15 mM K^+ by 750 and 1500 mg L^{-1} AsA were about 32% and 56%, respectively while increase in shoot length was 47% under both concentrations. Under 30 mM K^+ treatment and 750 and 1500 mg L^{-1} AsA, the leaf area increased by about 49% and 63%, respectively while the shoot height increased by 48% in both concentrations.

Effect of K^+ and AsA on photosynthetic pigments

Results indicated that photosynthetic pigments changed significantly in plants treated with K^+ (Fig. V). Under K^+ deficiency, the amount of chlorophyll a, b, and carotenoids decreased about 18%, 23%, and 16%, respectively compared to the control plants. This decrease was almost 19% of the total chlorophyll.

K^+ treated plants (15 and 30 mM) showed an almost 35% increase in chlorophyll a, 71% in chlorophyll b, and 28% in carotenoids compared to the control plants; however, total chlorophyll increased by almost 44%.

Two concentrations of AsA had significant effects on the amount of photosynthetic pigments. Treatment with 750 mgL^{-1} AsA resulted in increases by about 13%, 37%, and 17% in the chlorophyll a, b, and carotenoid, respectively. Foliar application of 1500 mgL^{-1} AsA showed about 33%, 68%, and 37% increases in chlorophyll a, b, and carotenoid contents, respectively. The amount of total chlorophyll increased by almost 20% and 44% in the plants treated with 750 and 1500 mgL^{-1} AsA, respectively.

The interaction effect of K^+ and AsA had a significant effect on photosynthetic pigments. There were gradual increases by about 48% and

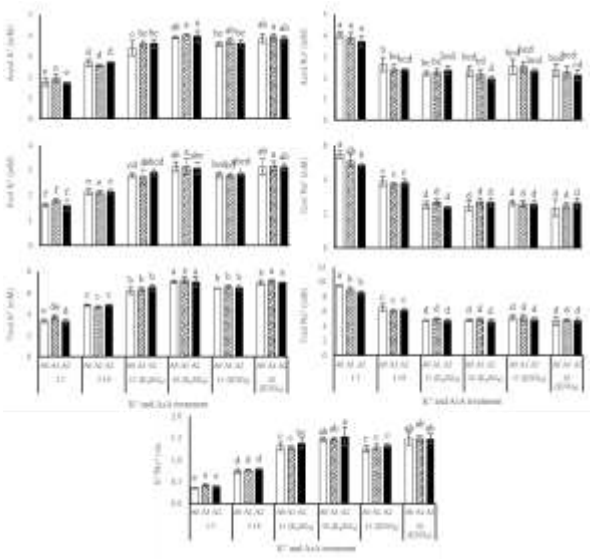

Fig. VII. Effect of K^+ and AsA on Sodium (Na^+) and potassium (K^+) content (mM) in 90-day old *C. roseus* plants; abbreviated letters A0, A1, and A2 are used for 0 (control), 750, and 1500 mg L^{-1} AsA, respectively. These concentrations were sprayed on leaf surfaces until dripping. Potassium (K^+) concentrations (mM) was used as either KNO_3 or K_2SO_4 in Hoagland's nutrient solution. Values are the means of the three replicates and different letters indicate the significant differences among the mean values based on Duncan's multiple range test ($p \leq 0.05$). The bar lines represent the standard deviation (SD).

71% for chlorophyll a and b, respectively in the plants treated with 15 or 30 mM K^+ with 750 or 1500 mgL^{-1} AsA, respectiveloy. Carotenoid

contents increased by about 37% in the plants treated with 15 or 30 mM K^+ with 750 mgL^{-1} AsA, while they increased by almost 56% in 15 or 30 mM K^+ with 1500 mgL^{-1} AsA. In the excess of K^+ (15 or 30 mM) with the foliar spraying of of AsA, the amount of total chlorophyll increased by 55% compared to the control plants.

Effect of K^+ and AsA on total free amino acids

Results showed that K^+ deficiency led to an increase in total free amino acids while the amount of total free amino acids decreased with an increase in K^+ concentration (Fig. VI). In this regard, the amount of total free amino acids in K^+ deficiency was 3 times higher than that in control plants, but in the excess of K^+ it decreased about 2 times compared to the control plants.

The foliar spraying of AsA (750 or 1500 mg L^{-1}) led to a decrease in total free amino acids by almost 1.5 times in control plants (3.16 mM K^+ with no AsA). Also, spraying AsA in combination with K^+ (15 or 30 mM) led to further reduction (about 4 times) in total free amino acids.

Effect of K^+ and AsA on sodium (Na^+) and potassium (K^+) contents

The content of K^+ and Na^+ are quantified in Fig. (VII). The highest and the lowest potassium contents were observed in both aerial parts and roots of plants under 30 mM and 1.5 mM K^+. The K^+ content increased shoot height and root length by 45% and 46%, respectively under 30 mM K^+, and decreased shoot height and root length by 34% and 25% under 1.5 mM K^+ treatment compared to the control plants. The total amount of K^+ decreased by about 30% under K^+ deficiency while it increased by 28% under 15 mM and 45% under 30 mM K^+.

In contrast, the amount of Na^+ increased in K^+ deficient plants about 53% for aerial parts and 40% for roots compared to the control plants. The amount of Na^+ decreased by 53% under 15 and 30 mM K^+ for roots, but there were no differences between Na^+ contents in control plants and plants treated with 15 or 30 mM K^+ for aerial parts. The total amount of Na^+ increased by almost 45% under K^+ deficiency but decreased about 28% in 15 or 30 mM K^+.

The ratio of K^+/Na^+ showed a gradual increase from K^+ deficient plants to plants treated with 30 mM K^+. In this case, the ratio of K^+/Na^+ in K^+ deficiency was 51% lower while it was 77% under 15 mM K^+ and 100% under 30 mM K^+ higher than control plants.

The data showed that ascorbic acid had no significant effect on K^+ and Na contents for aerial parts and roots, or the ratio of K^+/Na^+. Similarly, the interaction effect of K^+ and AsA had no significant effect on K^+ and Na^+ contents.

Discussion

Based on the growth curve, the diminishing growth phase happened on day 78, when the plant usually starts to produce secondary metabolites such as alkaloids instead of consuming its nutrients only for vegetative growth. Spraying with AsA was done on day 78, i.e. 10 days a precaution. Also, the sampling time was selected before the flowering phase. Furthermore, we measured the total AsA in the leaves of control and treated plants. The AsA content in the treated plants was higher than that in the control plant. Treating grapes with AsA was reported to increase leaf ascorbic acid content (Zonouri et al., 2014) and the internal AsA increased in *Chenopodium quinoa* when AsA applied (Aziz et al., 2018). In fact, many studies have reported that foliar application of AsA can effectively improve the plant AsA content (Ghorbanli et al., 2012; Singh and Bhardwaj, 2016).

Results indicated that growth and physiological parameters are affected by the interaction of potassium (K^+) and ascorbic acid (AsA) as well as the concentration of separate treatments.

The effect of nutrient elements at different levels on the properties of medicinal plants has been explored (Nagaveni et al., 2018). In this regard, the effect of different levels of essential macro-nutrients in the increasing of plant height, leaf area, and yield was reported (Nagaveni et al., 2018). K^+ is an essential cation that plays an important role in growth and developmental, physiological, and biochemical processes (Tang et al., 2015). A significant correlation was found between the amounts of K^+ and plant growth and physiological parameters

(Besford, 1978). K^+ deficiency and the excess of K^+ control the plant growth. Thus, managing of K^+ fertilizer is very important to reach optimum plant growth (Iqbal and Hidayat, 2016). Several studies indicated that K^+ deficiency alters the morphological indices (Gerardeaux et al., 2009) and accumulation of biomass (Makhdum et al., 2007). K^+ deficiency decreased leaf area and dry matter accumulation (Zhao et al., 2001) and caused a lower growth rate (Clavijo-Sánchez et al., 2015). It was observed that the K^+ deficiency in the vegetative phase of cotton (*Gossypium hirsutum* L.) reduced the production of plant dry matter and leaf area and resulted in a reduction in plant growth (Gerardeaux et al., 2009). Also, K^+ deficiency decreased root yield and total biomass productivity in sweet potato (Tang et al., 2015). In contrast, an adequate level of K^+ can enhance the total dry mass accumulation in plants (Egilla et al., 2001). It was indicated that K^+ nutrition increased plant leaf area and total dry mass (Lindhauer, 1985). Increasing the amount of K^+ significantly increased fresh and dry weights in shoots (Arafa et al., 2011) and leafs (Jin et al., 2011) as well as height and yield of *Zea mays* L. (Ortas, 2018), *Capsicum annuum* L. (Hussein et al., 2012), bushbean (Islam et al., 2004), and potato (*Solanum tuberosum* L.) (Zelelew et al., 2016). K^+ helped to increase the utilization of carbohydrates, and this resulted in increasing the leaf area and dry matter accumulation in *Brassica napus* L. (Cheema et al., 2012). It was reported that improving plant yield is due to the optimum level of K^+ for each plant (Huda et al., 2010). Also, maximum plant height, leaf area, and root length were obtained under the adequate K^+ treatment (Huda et al., 2010; Hussain et al., 2011; Kwizera et al., 2019). In fact, K^+ is a major nutrient for root development and maximum root length was obtained by increasing the K^+ level (Khan and Sajid, 2007). In this study, an increase was observed in the pattern of growth parameters such as wet and dry weight as well as leaf area, and plant height under K^+ deficiency to the excess of K^+. The lowest amounts of these parameters were related to the K^+-deficient plants (1.5 mM K^+) while the highest amounts were observed under the excess of K^+.

K^+ promotes the growth of meristematic tissue and activates some enzymes (Bhandal and Malik, 1988). The effects of K^+ on growth might be attributable to changes in activities of K^+-dependent enzymes (Kwizera et al., 2019). The effectiveness of a high level of K^+ is related to the role of K^+ in promoting enzyme activity and enhancing the translocation of assimilates and protein synthesis (Devlin and Witham, 1986). Also, K^+ results in improved cell membrane stability that is essential for cell, shoot, and root elongation. So, K^+ increases plant total dry mass production (Wang et al., 2013). In addition, K^+ is essential for the translocation of photo-assimilates in root growth (Römheld et al., 2010). Increasing K^+ increases root water uptake and consequently root surface, promoting root growth (Römheld et al., 2010).

In addition to using the K^+, it was observed that the foliar spraying of AsA leads to an increase in the wet and dry weight, leaf area, and plant height. Exogenous application of AsA in different ways such as foliar spray increases endogenous AsA (Chen and Gallie, 2004). It is well known that AsA has multiple developmental roles in plants (Ei-Tohamy et al., 2008; Herrera-Martínez et al., 2013). AsA plays an important role in cell growth and division, differentiation, and metabolism. Cell wall AsA and ascorbate oxidase have several important roles to control or increase growth. High ascorbate oxidase activity resulted in expanding cells quickly. (Smirnoff, 1996). Also, an increase in AsA concentration improves cell division (Gallie, 2013). AsA contributes to the cell cycle and regulation of cell growth and cell division (Tanaka et al., 2009). In addition, AsA increases the leaf area, which is responsible for the photosynthesis of the plant; changes in photosynthesis are a major control factor for plant growth (Tanaka et al., 2009). Therefore, AsA could be involved in a wide range of important functions in plants from the cell cycle, photosynthesis to growth regulation (Tanaka et al., 2009). It was indicated that the addition of AsA resulted in a significant increase in the growth parameters (Ei-Tohamy et al., 2008; Herrera-Martínez et al., 2013). Foliar application of AsA significantly increased plant height (Hussein and Alva, 2014), leaf area, fresh, and dry weights in sugarcane plants (Ejaz et al., 2012). The exogenous application of AsA enhanced biomass in the Brassica (Ameer et al., 2010), growth of Rosebushes (Herrera-Martínez et al., 2013), and

foliar plant growth that may contribute to increasing biomass and yield (Hussein and Alva, 2014).

The interaction effect of K^+ and AsA increased plant wet and dry weight and plant height as well as leaf area because of the additive effect on the creation and stability of optimal plant conditions. K^+ and AsA act as cofactors in some enzyme activities (Prajapati and Modi, 2012). The optimum concentration of K^+ and AsA creates an optimum condition for enzymes of metabolic pathways for better condition in the plant metabolism. Furthermore, its effect on the durability of photosynthetic pigments as well as creation of optimal photosynthetic conditions that lead to the further increase in the production of plant nutrients, and result in increased plant growth.

K^+ plays a key role in regulating the opening and closing of stomata, and K^+ deficiency induces stomatal closure and inhibits photosynthetic rates (Jin et al., 2011). Also, the leaf area was reduced under K^+ deficiency. Reduction of leaf area reduced photosynthetic rate per leaf which resulted in a decrease in plant growth (Pettigrew, 2008). Also, the leaf surface area and sunlight interception significantly reduced under K^+ deficiency, so K^+ controls photosynthesis through sunlight interception (Bednarz et al., 1998) as well as leaf area. In addition, it is well established that the amount of photosynthetic pigments is affected by K^+ concentrations (Hussein and Alva, 2014; Hu et al., 2017). There was a significant reduction in chlorophyll a, b, and total chlorophyll content under K^+ deficiency (Hu et al., 2017). In contrast, the total chlorophyll content was significantly higher in K^+-treated plants (Jin et al., 2011). The amount of chlorophyll significantly increased in K^+ treatment in *Capsicum annuum* L. (Hussein et al., 2012), and the highest concentration of leaf pigments in pea plants were obtained under K^+ treatment (Shafeek et al., 2005). Also, the amount of carotenoids correlated with the chlorophyll in all leaf sections and chlorophyll and carotenoid contents were higher in the highest concentration of K^+ (Sideris and Young, 1945). So, K^+ treatment resulted in an increase in the amount of carotenoid as well as chlorophyll (Fayez and Bazaid, 2014). We observed that different concentrations of K^+ had different effects on the leaf pigments, so that K^+ deficiency led to a decrease in the amount of chlorophyll a, b, total chlorophyll, and carotenoids while the excess of K^+ resulted in an increase in their contents.

Chlorophyll degradation happened under K^+ deficiency (Wall, 1940; Hasanuzzaman et al., 2018). In contrast, the excess of K^+ reduced electrolyte leakage which in turn, resulted in the increased total chlorophyll content (Zhao et al., 2001). The effective transfer of photosynthetic electron significantly increased photosynthesis ability that occurred in K^+ sufficient plants (Ei-Tohamy et al., 2008; Jiang et al., 2008). Additionally, K^+ deficiency led to chlorophyll reduction that decreased the photosynthetic rate and restricted the translocation of photosynthesis from leaves, increasing sucrose and starch in leaves which led to a significantly lower amount of chlorophyll and development of leaf. As a result, under K^+-deficiency, photosynthetic rate, leaf area, and dry matter accumulation decreased significantly (Zhao et al., 2001). Furthermore, K^+ deficiency led to the destruction of PSII that is caused by excessive solar energy absorption. So, adequate K^+ maintains optimum photosynthesis and thus increases plant growth (Wang et al., 2015).

A high concentration of AsA is very important to neutralize oxidative stress as well as regulate other metabolism processes in plants. The reduction of pigment contents is due to the low rate of synthesis or rapid degradation of pigments occurring under stress conditions like K^+ deficiency. As stress condition induces gene expression of chlorophyllase, it leads to increasing of chlorophyllase activity which is a reason for the decreasing of chlorophyll concentration (Silva et al., 2007). Also, the decrease in chlorophyll under stress is related to chloroplast damage by reactive oxygen species (ROS) (Jung, 2004). AsA significantly protects plants from stress. AsA is a detoxifier of active radical compounds, so it can prevent chlorophyll degradation, and indirectly enhance the amount of chlorophyll in leaves (Bybordi, 2012). The higher concentration of AsA reduced ROS accumulation, and this reduction protected the function of leaves in rice (Zhang et al., 2018). It is possible that AsA with its anti-oxidative properties prevents degradation of

chlorophyll (Zonouri et al., 2014) that is along with reducing ROS (Zhang et al., 2018), and resulted in an increase in the amount of chlorophyll. Our data showed that AsA led to a significant increase in chlorophyll a, b, total chlorophyll, and carotenoid contents especially in the lower concentrations of K^+. In this regard, it is reported that AsA is an important compound for increasing the amount of photosynthetic pigment (Herrera-Martínez et al., 2013). Chlorophyll a and b were positively affected by AsA in canola (Bybordi, 2012). It was observed that the foliar application of AsA modified the harmful effects of stress due to affecting several parameters like total chlorophyll content, protein synthesis, photosynthesis, and plant growth. It was reported that AsA treatment caused an increase in the amount of carotenoids in grapes (Zonouri et al., 2014). The amount of chlorophyll and soluble proteins correlated with the concentration of AsA positively.

Amino acids are precursors for the biosynthesis of proteins and secondary metabolites in various plants (Bennett and Wallsgrove, 1994). The amount of total free amino acids is a result of the synthesis and degradation of amino acids and protein (Obata et al., 2012). Total free amino acids may change in response to environmental factors and are very important in protein biosynthesis and different metabolic pathways (Obata et al., 2012). Total free amino acids accumulated sharply in K^+ deficiency because amino acids could not be successfully used in synthesizing proteins. So protein degradation results in forming free amino acids (Hu et al., 2016). In our study, tdifferent concentrations of K^+ had different effects on total amino acid content. K^+ deficiency increased the amount of total free amino acids while the excess of K^+ decreased this trait. In line with our results, free amino acid content increased under K^+ deficiency in cotton leaves (Hu et al., 2017), corn leaves (*Zea mays* L.) (Hsiao et al., 1970), tobacco (Koch et al., 1974), and barley (Helal et al., 1979). Since an increase in the amount of free amino acid means a decrease in the protein content, decreasing protein content in K^+ deficiency leads to a reduction in growth parameters like plant weight. On the other hand, as increasing the amount of K^+ decreases the amount of total free amino acids, an increase in plant weight and growth happened that can be due to an increase in plant protein content.

Decreasing free amino acids can be a result of further production of proteins and secondary metabolites in AsA-treated plants. Under stress conditions, the transcription of the genes encodes the enzymes involved in the arginine biosynthesis (Kovács et al., 2012) and asparagine synthase in sunflower (Herrera-Rodríguez et al., 2007) and wheat (Wang et al., 2005) increases while enzymes involved in the degradation of free amino acids decrease. Also, increasing the level of amino acids is related to oxidative stress in plants and is a result of oxidation of proteins, even in the presence of endogenous antioxidants (Stadtman and Oliver, 1991). Antioxidant compounds can modulate the free radical compounds that oxidize proteins (Gebicki et al., 2010). AsA is one of the most important antioxidants that reduce oxidizing radicals significantly (Domazou et al., 2009). So, it can effectively reduce total free amino acids (Gebicki et al., 2010). In this experiment, spraying AsA reduced the amount of total free amino acids. Similar to our results, some amino acids decreased under exogenous AsA in spinach (*Spinacia oleracea* L.) (Min et al., 2020).

In addition, the interaction effect of K^+ and AsA decreased the amount of total free amino acids. Different proteases break proteins into amino acids (Hildebrandt et al., 2015). AsA acts as a cofactor of enzymes that involve in protein biosynthesis (Herrera-Martínez et al., 2013) as well as an H^+ donor for various enzymes (De Carolis et al., 1990). Spraying AsA on the leaf surfaces may result in preparing a source of H^+ donor for these enzymes; consequently, besides the positive effects of K^+, these essential enzymes can regenerate so quickly that they result in producing more proteins, followed by a decrease in the amount of total free amino acids.

K^+ adjusts the balance between cations and anions in some processes of metabolism pathways. The protection of the osmotic potential of plant vacuoles are achieved by K^+ and other cations such as Na^+. The substitution of K^+ by Na^+ has very important relationship with the management of treatment and plant growth in plant physiology. An array of research has been done on the replacement of Na^+ with K^+ in plant

nutrition. For some of its functions, Na^+ has the potential (Wakeel et al., 2011). For normal function and plant growth, control of K^+ concentration and K^+/Na^+ ratio is very important (Wang et al., 2013). The exogenous K^+ decreases the uptake of harmful nutrients that leads to increase in plant yield (Ganie et al., 2017). K^+ maintains ion homeostasis and enzymatic activities, so it acts as a crucial protector under different stress conditions (Hasanuzzaman et al., 2018). Na^+ competes with K^+ for binding sites and disturbs the plant metabolism (Wang et al., 2013). Maintaining the cellular K^+ content and a high K^+/Na^+ ratio is so important for plant growth. It is achieved by increasing the K^+ content in the plant and is gained by a higher concentration of K^+ and results in reducing the Na^+ concentration as well as increasing the K^+/Na^+ ratio (Flowers et al., 2015).

Our results indicated that increasing the concentrations of K^+ in the treatment resulted in rising the amount of K^+ in the plant while the amount of Na^+ decreased. Also, the K^+/Na^+ ratio was significantly higher than K^+-deficiency under the treatment involving the excess of K^+. Similar to our results, treating plants with K^+ showed an increase in the concentration of K^+ as well as height and yield (Ortas, 2018). It is observed that salinity resulted in an increased Na^+/K^+ ratio, and K^+ treatment led to a decrease in this ratio; consequently, the K^+/Na^+ ratio increased (Fayez and Bazaid, 2014). In addition, external applications of K^+ by adjusting the tissue ionic balance reduced the Na^+ uptake in peanut (*Arachis hypogaea* L.) (Chakraborty et al., 2016). It was observed that Na^+ was replaced with K^+ under K^+ treatment in tomato plants. The plants absorbed K^+ instead of Na^+; therefore, the highest K^+/Na^+ ratio was achieved (Besford, 1978). In contrast, K^+-deficiency decreased the K^+ content in the leaves and roots that resulted in inhibiting plant growth (Pi et al., 2014). These factors in K^+-deficient plants lead to a further reduction of plant growth parameters while the optimum concentration of K^+ promotes plant growth. The combination of the above factors can eventually lead to decreasing and increasing plant growth parameters under K^+ deficiency or the excess of K^+, respectively.

In conclusion, under K^+ deficiency, different factors such as reduction of the activities of K^+-dependent enzymes (Kwizera et al., 2019) results in a decrease in cell elongation (Wang et al., 2013) and disorder of translocation of photo-assimilates (Römheld et al., 2010) caused a decrease in growth parameters. Also, inhibiting photosynthetic rates by stomatal closure (Jin et al., 2011), and the leaf area (Pettigrew, 2008), degradation and reduction of photosynthetic pigments (Hasanuzzaman et al., 2018) and disruption the photosynthetic organ (Zhao et al., 2001) are other factors to decrease growth parameters. Besides, the failure of protein synthesis using amino acids and degradation of proteins to produce free amino acids (Hu et al., 2016) result in the reduction of plant growth and weight. The excesses of K^+ and AsA promote the enzyme activity, improve cell membrane stability, translocation of photo-assimilates, and the leaf area, and prevent degradation of photosynthetic pigments. Therefore, K^+ and AsA control photosynthesis rate to optimum condition for plant growth parameters. In addition, K^+ and AsA involve in maintaining the balance between protein and amino acid synthesis and break down. So, increasing photosynthetic pigments lead to an increase in photosynthesis rate with a decrease in free amino acids, which is a sign of the synthesis of protein and other metabolites to promote more plant growth. Also, because of K^+ and Na^+ competition for normal function and plant growth, controlling K^+ concentration and K^+/Na^+ ratio is very important. Decreasing harmful nutrients in plant leads to increasing wet and dry weight, and ultimately increase plant growth parameters. Therefore, the optimum concentration of K^+ and AsA creates an optimum condition for enzymes in the metabolic pathways of plant metabolism and ultimately increases plant growth parameters.

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