

The effect of gibberellic acid application methods on morphophysiological traits, biochemistry indices, and fruit number of Jerusalem Cherry (*Solanum pseudocapsicum* **L.)**

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Abstract

A greenhouse experiment was performed on Jerusalem cherry treated with gibberellic acid (GA3) in four levels (0 (control), 100, 200, and 400 mg/lit) and three types of application method (foliar, drip treatment, and foliar + drip treatment). Results showed significant reciprocal effects of experimental factors on plant height, lateral shoot length, lateral shoot number, fruit number, root length, root wet weight, stem wet weight, chlorophyll (a, b, total), pigment function, chlorophyll index, and reducing sugars. Simultaneous application of foliar spray and drip application method $+ GA_3$ increased carotenoid and leaf area ratio compared to the use of each treatment alone. The highest amount of chlorophyll a (19.75 µg/ml), chlorophyll b (4.63 µg/ml), total chlorophyll (25.27 µg/ml), and the total amount of pigments (27.67 µg/ml) were obtained by leaf foliar spray and drip treatment with 400 mg/lit, drip treatment with 200 mg/lit, and foliar spray and drip application method with 200 mg/lit concentration. The use of GA_3 increased the number of fruits, pigment performance, and reducing sugars. Combined GA₃ application methods of leaves foliar spray + drip application) resulted in better performance than each method alone. Therefore, combined application method is recommended for GA³ treatment to improve Jerusalem cherry's morphological and photochemical characteristics.

Keywords: chlorophyll, application method, gibberellic acid, Jerusalem cherry, reducing sugars

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Introduction

The Jerusalem cherry with the scientific name of *Solanum pseudocapsicum* L. belongs to the Solanaceae family. It is a decorative perennial old

plant, native to Africa, known as winter cherry, and is considered for its beautiful fruit (Ghasemi et al., 2013). Nowadays, ornamental plants are a multibillion dollar market worldwide markets, and this makes most countries pay attention to these plants (Ghasemi et al., 2013). Using different breeding methods, researchers seek to increase the quality and quantity of flowers and

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ornamental plants. One of the breeding tools is plant growth regulators including gibberellins, which have a broad application in ornamental plants (Shahdadnezhad et al., 2019). Gibberellic acid plays an essential role in plant growth and development. Studies on growth regulators such as gibberellic acid (GA_3) show that growth regulators can enhance pigment levels, such as carotenoids (Hyun Jin et al., 2007).

Treatment of Gladiolus plants with gibberellin was efficient at the beginning of rooting and increased the plant height by 16.51% and 3.67%, respectively (Sharmila et al., 2012). Danaee et al., (2011), found that $GA₃$ in a concentration of 50 mg/lit increased total soluble solids and water content of the flower and improved the quality and vase life of Gerbera. Rouhi and Esmaeilzadeh (2013) also reported that 450 mg/lit concentration of GA³ had the highest effect on the quantitative and qualitative indices of the pomegranate fruit. In another experiment on the quantity and yield of the grapes, it was found that the plants treated with GA₃ recorded the highest fruit weight, single grape diameter, and overall performance compared to other treatments and control (Sangeetha et al., 2015). Salehi Sardoei's (2014) showed that foliar treatment of leaves and soil treatment of GA₃ had more effects on Aloe vera L. The present study aims to investigate the effects of methods of gibberellic acid application on some morphological characteristics, reducing sugars, flower pigments, and the quality of the ornamental plant Jerusalem cherry.

Material and Methods

Plant materials and culture conditions

This study was carried out to investigate the effect of $GA₃$ on the morphological characteristics and photosynthetic pigments of Jerusalem cherry plant in the form of a factorial experiment using complete random block design (CRBD) in three iterations. Average daily and night temperatures in greenhouses during late fall and winter were 2 \pm 22 °C and 2 \pm 18 °C, respectively, and the relative humidity of the greenhouse was over 70 %. Plant nutrition was carried out equally from micro bud feeding solution (Aras Baran Agricultural Company) by 3% concentration in 4 consecutive

weeks containing nitrogen 8%, phosphorus 4%, potassium 5%, and microelements including iron, copper, zinc, manganese, molybdenum, and bor.

Application methods

In this experiment, Jerusalem cherry's seedlings with 1±6 leaves were treated with gibberellic acid (Sigma Company) at concentrations of zero (control), 100, 200, and 400 mg/lit. There were three application methods in the study, namely leaf foliar spray, drip treatment, and combined application of foliar spray + drip in three stages of leave foliar spray (the potting soil was wet), soil treatment (at each stage, the plants were irrigated with 40cc), and at intervals of 15 days (Salehi et al., 2014). The foliar spray of the control plants was carried out with distilled water.

Measuring physiological parameters

The photosynthetic pigment measurement was followed up according to the Lichtenthaler method (1987). The light absorption samples were carried at wavelengths of 663 (chlorophyll a), 646 (chlorophyll b), and 470 (carotenoids) nm by spectrometers device. Finally, the photosynthetic pigments in terms of microgram/mL were assayed using the following equations.

The chlorophyll contents of the young leaves were assayed using a chlorophyll meter device (model CL-01) in the early morning hours (Ghasemi et al., 2016).

Somogy (1952) method was used to measure the levels of reducing sugars. For this purpose, 0.02 g of aerial parts of the plant was ground with 10 mL distilled water in a porcelain mortar. The contents

Table 1 Analysis of variance of gibberellic acid and its application methods affecting growth indices characteristics of Jerusalem cherry

*, ** and, ^{ns} show significant at P<0.05, P<0.01, and non- significant, respectively.

were then transferred to a small beaker and placed on the electric hot plate to heat. As soon as reaching the boiling point, the heat was cut off, and the extract was passed through Whatman's filter paper grade one. Two ml of each of the prepared extracts were transferred to the test tube, and after adding 2 ml of copper sulfate solution, the tubes were sealed with cotton and placed in a hot bath at 100 ℃ for 20 minutes. At this stage, the $Cu₂⁺$ is converted into $Cu₂O$ by reduced monosaccharide aldehyde, and Rubiginous is left at the bottom of the tube. After the tubes were cooled, two ml of Phospho molybdic acid solution was added to them, and after a few moments, a blue color appeared. The test tubes were vigorously shaken so that the color was uniformly propagated within the test tube. The spectrophotometer determined the adsorption intensity of solutions at 600 nm wavelength, and using a standard curve reduced sugars were calculated.

Measuring vegetative indices

The seedlings were removed from the pots 180 days after treatment, and different parts of the plant including root, stem and, leaf were separated from each other and morphological features such as plant height (measured by ruler and cm), the number of side shoots, the number of fruit (determined by counting at the time of harvest), side branch length and root length (measured by a ruler in mm), and root and shoot

wet weights were measured by P<0.01 error scale in grams. The leaf area measurement device was used to measure leaf area.

To analyze the results, a completely randomized design was used with ANOVA. The data from measurement of the parameters were compared using SAS software, and the means were compared using the Duncan test at P<0.05.

Results

The results obtained from the variance analysis of the data showed that the main effect of $GA₃$ was the reciprocal impact of $GA₃$ in hormone consumption at the level of significant of P<0.01 on plant height (Table 1). The comparison of the reciprocal averages showed that the maximum plant height (86.33 cm) was obtained at the concentration of 400 mg/lit of drip treatment, which was followed by all levels of $GA₃$ use (Table 2). The number of side shoots of Jerusalem cherry was affected only by interaction effects of Hormone and application method of hormone, according to Table 1. Maximum numbers of side branches (67.219 and 218.218 cm) were obtained from the control treatment and foliar spray with 400 mg/lit GA3 (Table 3). The lateral shoot length was also affected by the interaction effects of GA₃ and the method of hormone application. The highest lateral shoot length (58.10 cm) was obtained from the control treatment and foliar leaf spray + drip treatment.

Table 3

Analysis of the effect of gibberellic acid and its application method on some physiological and biochemical properties of a Jerusalem cherry

*, ** and, ^{ns} show significant at P<0.05, P<0.01, and non- significant, respectively.

As shown in Table 2, 400 mg/lit GA_3 and foliar spray method increased the number of fruits, which was significantly different from other concentrations and methods. The results of the analysis of the variance corresponding to the root length indicated the significance of the main effects and the interaction effects of the hormone and the application method of the gibberellic acid hormone (Table 1). According to Table 1, mean impacts of the combined use of the foliar spray

and drip treatment of gibberellic acid, with a concentration of 100 mg/l, had the maximum root length. On the contrary, the lowest root length was observed in the control treatment (Table 2). In line with the significant interaction effects of GA3, application of GA³ 400 mg/lit treatment, and drip treatment resulted in the highest fresh weight of shoot (299.58 g) as shown in Table1. Fresh root weight was also affected by the main effects and interaction effects of $GA₃$ and application

GA ₃	Treatments	Chlorophyll a	Chlorophyll b	Total chlorophyll	Pigment function	Leaf chlorophyll
(mg/lit) Control	Foliar spray	(mcg/ml) 13.22^{f}	(mcg/ml) 3.36 ^d	(mcg/ml) 18.97 ^{ef}	(gr/per plant) 18.41 ^g	(SPAD) 45.68 ^b
	(FS) Drip treatment (DT)	14.35^{fe}	3.51 ^{cd}	25.27a	19.92fg	38.27^e
	$FS + DT$	17.40bc	3.81^{bc}	19.25 ^{ef}	23.66^{bc}	37.837 ^e
100	Foliar spray (FS)	17.37bc	3.44 ^{cd}	21.40cd	23.38cd	52.94 ^a
	Drip treatment (DT)	16.26 ^{cd}	3.85^{bc}	23.92ab	$22.74c-e$	39.67de
	$FS + DT$	19.34a	3.75^{b-d}	18.37^{fg}	25.87ab	45.30 ^b
200	Foliar spray (FS)	15.76 ^{de}	3.49 ^{cd}	21.21 ^{cd}	21.60^{c-f}	43.70b-d
	Drip treatment (DT)	15.61 ^{de}	3.35 ^d	16.58^{g}	21.18^{d-f}	$40.32c-e$
	$FS + DT$	20.64a	4.63 ^a	17.86^{fg}	27.67 ^a	31.89f
400	Foliar spray (FS)	14.82de	3.55 ^{cd}	23.09bc	20.52^{e-g}	44.90bc
	Drip treatment (DT)	17.80 ^b	3.60 ^{cd}	20.81 ^{de}	23.78 ^{bc}	38.38 ^e
	$FS + DT$	19.75 ^a	4.17^{b}	20.35^{de}	26.53 ^a	39.32de
	\mathfrak{Z} $2.5\,$ Carotenoids (µg/ml) c $\sqrt{2}$ $1.5\,$ 1 0.5 $\mathbf{0}$ $\boldsymbol{0}$	a 100 200	$\mathbf a$ b 400	Carotenoids (µg/ml) 2.6 2.5 2.4 2.3 b 2.2 2.1 $\overline{2}$ Spray	a b Spray+Drip Drip treatment treatment	$\mathbf b$
Gibberellic acid levels (mg/l)				Gibberellic acid use method		

Table 4 Effects of study treatments and their interactions on chlorophyll contents of Jerusalem cherry

Fig. I. Comparison of the mean effects of the gibberellic acid (a) and the hormone application method (b) on carotenoid contents

techniques (Table 1). The highest root fresh weight was obtained in the combination of foliar spray treatment and drip treatment with 100 mg/lit GA_3 (Table 2).

Analysis variance in Table 3 showed that the interaction effect of the experimental treatments had a significant impact on chlorophyll a and b, the total chlorophyll, pigments function, and the degree of leaf chlorophyll. According to the mean comparison Table 4, the highest chlorophyll a content (64.20 µg/ml) and also chlorophyll b content (63.4 µg/ml) were obtained using combined treatment of leaf foliar spray and soil

application method with 200 mg per liter of $GA₃$ (Table 4). Hence, the maximum amount of total chlorophyll was obtained from the control treatment, and the use of the soil method, which showed a significant difference from the other treatments of the study (Table 4).

Carotenoid content was affected only by the main effects of GA_3 and hormone consumption methods. Carotenoids increased as compared with control treatment. There was no significant difference between 200 and 400 mg/lit concentrations of GA₃ (Fig. I. a). The simultaneous foliar spray and drip treatment procedure among

Fig. II. Comparison of the main effects of the gibberellic acid (a) and the hormone application method (b) on leaf area; in each column and each test factor, the same letters show no significant difference based on Duncan Multiple Range Test (P< 0.05).

Fig. III. Mean comparisons of the main effects of the gibberellic acid and its application method on reduced sugars of the Jerusalem cherry

the hormone-consuming methods had the most significant effect on the carotenoid contents (Fig. $I. b$).

The results of the variance analysis of the data on the pigment function indicated significant main effects and the interaction effects of $GA₃$ and the method of using GA_3 (Table 3). Table 3 shows that the maximum performance of pigments (27.67 µg/ml) was obtained from combined use of foliar spray and drip treatment with 200 mg/lit GA₃ (Table 4). In addition, only the main effects of GA³ and hormone application method were significant at the probability level of P<0.01 (Table 3).

Regarding the significance of the main effects, GA³ increased the leaf area and the highest value was obtained by using the 400 mg/lit GA3, which was not significantly different from 100 and 200 mg/lit growth hormone (Fig. II. a). Also, the highest value of the leaf area was obtained by foliar spray and drip treatment of the GA_3 (Fig. II. b). The highest leaf chlorophyll content (52.94 µg/ml) was related

to 100 mg/lit concentration of GA_3 (Table 4). The measurement results of this biochemical trait showed that the highest reducing sugar contents of 82.71 g and 75.41 g fresh weight was obtained from 100 mg/lit GA_3 through drip treatment and 200 mg/lit GA_3 through foliar spray application, respectively, showing an increase by more than 20% in comparison with the control (Fig. III).

Discussion

The effect of GA_3 along with application methods was evaluated by examining a wide range of quality and quantity attributes in Jerusalem Cherry. The role of GA_3 and its application types on determining side shoot length was investigated. The shoots of this ornamental plant were elongated to 218 cm under 400 ppm of $GA₃$, whereas all integrated treatments have increased shoot length even in a low dose of gibberellin (Table 2). Neomayer et al. (1987) reported that high concentrations of $GA₃$ resulted in excessive lengthening of the shoot of Iranian violets

plantlets and thus decreased the quality of plantlets. All known gibberellic acid is effective in growth stimulation, shoots length growth, cell division, or both (Arteca, 2010). It was reported that foliar spray of GA_3 at concentrations of 100 mg/lit in Polianthes tuberosa increased flower stalk length and had a significant effect on the fresh and dry weight of the plant (Panwar et al., 2006).

Plant height and wet weights of stems and roots were significantly affected by the interaction of $GA₃$ and application methods (Table 2). In our study, we used the GA_3 400 ppm by drip treatment, which is 86.33 cm for the maximum height. Also, the best fresh stem and root weight was obtained from GA_3 400 ppm + drip method (299.58 g) and foliar spraying with $GA₃$ 100 ppm + drip application (68.35 g). Foliar spray of Anthurium with GA_3 200 mg/lit increased plant height (Dhaduk et al., 2007). In an experiment, Edalati Marfe et al. (2013) reported that the fresh weight of Alstroemeria flowers increased significantly by increasing the concentration of $GA₃$ in the solution. Still, there was no significant difference between the concentration of 100 and 150 mg/lit of GA_3 . Foliar spray of GA_3 at a concentration of 300 mg/lit in parsley flower increased the height and number of branches per plant (Kishan et al., 2007). Another study showed a significant increase in plant height with an average of 5/69 cm in Anthurium plant obtained by the foliar spray of 500 mg/lit $GA₃$ (Dhaduk et al., 2007). In the iris flower, the application of 250 mg/lit GA_3 significantly increased shoot height (Al-Khassawneh et al., 2006). In the experiment on the lavender plant, the results showed that the plant height, wet and dry weight of leaves, and stem were highest in the treatment with 300 mg/lit of gibberellin (Hajisamadi Asl et al., 2011). The experimental results showed that by using $GA₃$ in the concentration of 300 and 500 mg/lit, the best results were obtained in terms of herb length by 101 and 97 cm. However, this treatment did not affect the number of branches in each cluster (Khuankawei et al., 2008).

Both GA₃ and its application methods influenced total chlorophyll which increased under the foliar spray and decreased under combined application

of foliar spry and drip treatment. The reduction of chlorophyll concentration is an essential factor in the rate of plant photosynthetic capacities, and increasing tension causes poor performance of the leaves in photosynthesis and aggravation of stress. Therefore, the decrease in vegetative traits can be attributed to the reduction of the photosynthetic rate to supply chlorophyll value (Abd El-Baky et al., 2008). One of the most important reasons for reducing chlorophyll is destruction by active oxygen. On the other hand, competition and overtaking the gamma-glutamyl kinase enzyme during stress from Glutamate Ligase Enzyme (the first enzyme of chlorophyll biosynthesis pathway) causes the glutamate precursor, more commonly used by amino acids, especially proline. Therefore, chlorophyll biosynthesis is subject to limitations (Gibson et al., 2000). There are conflicting reports in different sources on the use of $GA₃$ in photosynthetic processes, some of which represent an increase (Wareing et al., 1968) while others represent a decrease in the rate of these processes (Sanhla and Tuber, 1974).

The best chlorophyll function was obtained in $GA₃$ 400 mg/lit application (47 mg/lit) with a slight difference with other doses. In their experiment, Arteca et al. (1985) found that $GA₃$ stimulates the value of chlorophylls a, b, and total chlorophyll and photosynthetic rate, altering the shape and structure of plastids, and it could change the amount of Rubisco enzyme activity in vitro. These results were consistent with those of Mynett et al., (2001), who studied the effects of $GA₃$ on the leaf chlorophyll index of Freesia. $GA₃$ plays a structural role in the chlorophyll membrane and stimulates photosynthesis (Janowsk and Jerzy, 2003). Pigment function is an important factor in assessing the effects of hormones. In this study, we found that this value increased directly with increasing levels of the hormone as the highest content was obtained in the integrated treatments (Hormone and application method) 200 and 400 GA³ with the amount of 27.67 and 26.53 g/per plant. Studies on growth regulators, such as GA3, indicate that they can increase the number of popular pigments such as carotenoids (Glick et al., 2007; Hyun Jin et al., 2007; Kim et al., 2006). In a study, Kanjilal and Singh (1998) found that chlorophyll content increased under different

gibberellic acid concentrations. The use of $GA₃$ has led to an increase in anthocyanin content of grapes (Peppi et al., 2006).

Leaf area as an important morphological feature was measured in this study. GA₃, particularly in combined application (foliar spray + drip treatment) increased the leaf area of the plants under study. The experiment by Mohammadi et al., (2013) on the Persian cyclamen showed the concentration of 200 mg/lit $GA₃$ compared to the control treatment increased leaf area while higher concentrations had a negative effect on the growth of leaves.

Reduced sugars were also affected by $GA₃$ and application method. The reduced sugars in the drip treatments with 100 mg/lit $GA₃$ increased by almost 27/2% compared to control (Fig. III). The use of $GA₃$ was reported to cause an increase in soluble sugars of spathe flower (Salehi et al., 2014) confirming the findings of the present study. The amount of sugar (total soluble solid) is one of the most important factors in determining growth. The higher the percentage of the carbohydrate material storage, the more plant growth (Arteca, 2010). Combination of biochemical and physiological factors along with the environmental effects determines the plant's function. In this study, there was a significant difference between treatments in terms of reducing sugars. Correlations between $GA₃$ concentration and reducing sugar contents of leaf tissue have been reported in spathe flower (Salehi Sardoei et al., 2016), Petunia (Hussaini et al., 2014), Parsley (Hosseini et al., 2015), and Catharanthus roseus (Baniasadi and Saffari, 2015). Plant growth regulators such as GA3 accelerate the construction of photosynthesis proteins, cause cell

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development in some tissues and organs, which increases the amount of soluble solids, transporting materials made from leaves to other parts of plant, and increase dissolved carbohydrates, which cause cell swelling and increasing the growth (Arteca, 2010).

Conclusion

The effect of GA_3 in three levels and its application methods on Jerusalem cherry was assessed. The findings indicated that cell division and differentiation by growth regulators can increase growth and development. Besides, it is very essential in pot plants, especially fruitlet vase life. Therefore, it is possible to help growth and development and increase the length of the flowering period by using growth regulators because of their synergistic effect. Furthermore, plant growth regulators are more economical to create beautiful landscapes, especially in arid and semi-arid regions, which are regularly exposed to stress. From the results of this experiment, it can be concluded that the interaction effects of gibberellic acid and application method of this hormone have a significant impact on all traits (except carotenoids and leaf area). The results suggest that Jerusalem cherry plant is highly sensitive to gibberellic acid application method, so that the plant height and number and length of the stem increased considerably. The concentrations of 100 and 200 mg/lit gibberellic acid had the highest effect on quantitative and qualitative indices of Jerusalem cherry, and their simultaneous use of application methods had a better result than the single application of foliar spray or drip treatment.

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