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Research Paper

Investigation of hormone effects on reproductive properties of *Agave amica* in different grow condition

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Abstract

Agave amica (*Polianthes tuberosa* L) is one of the most important cut flowers in Middle East and the world and it is widely used in flower decoration due to its beauty and perfume [1]. To investigate the effects of plant density and Gibberellin treatment on tuberose bulbs, an experiment was conducted using a Split Plot based on Randomized Complete Block Design with three replications in the Jiroft city. In this experiment, plant density in three levels (10, 15, and 25 plants per m2) as the main factor and gibberellin concentrations in four levels (control, 100, 200, and 300 ppm) as a sub-factor were considered. The present study was performed in 12 treatments and three replications, which consisted of 36 experimental plots. The results showed that the studied indices were affected by the application of gibberellin and plant density treatments, except for the number of cromels per plant. Also, the highest values in the number of leaf per plant, inflorescence length and number of floret per inflorescence indices were obtained in the mentioned treatment, and the lowest amounts of them were observed in the density of 25 plants per m^2 × no application of gibberellin. Therefore, the best treatment for the cultivation of tuberose plant was the application of 300 ppm of gibberellic acid and the density of 15 plants per m^2.

Kaywords: Gibberellic Acid; Inflorescence Height; Planting Density; *Polianthes tuberosa*

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1. Introduction

Tuberose (*Polianthes tuberosa* L) is one of the most important of cut flowers in Middle East and the world, and because of its beauty and fragrance, it is popularly utilized in flower decoration industry (Kheiry et al., 2011). This plant, which grows mostly in tropical and subtropical regions, is native to Mexico and used for cultivation in gardens and parks. The essential oil of its flowers has been considered from ancient times and is now one of the most valuable components of the perfume industry in the world (Deheretogh LeNard, 1993). Different levels of ethylene reduce the longevity of the cut flower of tuberose while gibberellin application, especially when it treated for soaking bulbs before planting process, has more beneficial roles in increasing the longevity of cut flowers (Davis, 1988).

Kheiry et al. (2011) showed that application of gibberellic acid at the concentration of 500 ppm increased the length of flowering stem and the length of spike rachis, and also stimulated the emergence of the flowering stem. Also, the obtained results of the length of flowering stem measurement indicated that there was a significant difference between the studied concentrations and types of plant regulators at $p<0.01$. The highest length of the flowering stem (equal to 44.5 cm) was observed in 100 ppm of gibberellic acid × 500 ppm of benzyladenine interaction and the lowest length of the flowering stem (equal to 27.08 cm) was achieved in the no application of gibberellic acid × 1000 ppm of benzyladenine interaction. Further,

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the results showed that gibberellic acid had a principal role in increasing the length of flowering stems, but benzyladenine was an auxiliary factor which stimulated the length of flowering stems in appropriate concentrations of gibberellic acid application. The effect of gibberellin has been shown to increase the length of flowering stems in different plants. On the other hand, it has been proven that just its appropriate concentration was varied in different plants. Also, some studies have shown that the use of gibberellins has been effective in increasing the length of the flowering stem of cut flowers, and in most bulb flowers they can stimulate the flowering process and prevent the flower bud abortion (Chang et al., 2006). In the tuberose plant, the length of the flowering stem is an important qualitative factor that gibberellins can produce positive effects on this index. On the other hand, it has been reported that the effects of gibberellins were varied according to the type of plants, the used concentrations, and the time of their applications (Davis, 1988).

Some findings have shown that the content of free gibberellins increased in the bulbs of tuberose after the dormancy period (Chang et al., 2006). De and Dhiman (2001) showed that soaking the bulbs of tuberose plant for one hour under the concentrations of 50 to 100 ppm of gibberellin had positive effects on the vase life of the tuberose, inflorescence length, and floret opening. Nagarja and Gowda (1998), and Wiren and Nasau (2001) have stated that gibberellin and 6-benzyladenine can be displayed synergistic effects on vase life and opening the florets of cut tuberose flowers. In another study, tuberose bulbs were soaked in gibberellin and benzyl adenine solutions under concentrations of 50 and 100 ppm for 24 hours and then cultivated in an experimental farm. The results of the mentioned research shown that both treatments affected the growth and flowering characteristics of the plants. As well as, the above treatments were effective in accelerating the initial appearance of florets and speeding up the flowering process compared to control treatment. It was also observed that the highest quantity in inflorescence length was obtained under the concentrations of 100 and 50 ppm of present treatments in comparison with other treatments. Furthermore, it has been reported that the number of floret per each flower had increased by application of the gibberellin concentration of 50 ppm (Nagarja et al., 1999).

The aging process in plants involves a series of distinct symptoms, such as harmful, progressive, pervasive, and almost irreversible changes. Devendara and Nagda (1999) and Preeti and Gogoi (1997) reported that gibberellin at the concentration of 200 ppm accelerates the germination of tuberose bulbs and reduces the number of required days for germination from 20.83 days in the control treatment to 12.03 days. In this concentration, plant height, inflorescence length, and flower diameter were equal to 65.65, 103.96, and 67.3 cm, respectively, and the number of floret per each inflorescence was equal to 38.03 florets. In general, the results showed that mentioned indices had significantly increased in the concentration of 200 ppm of gibberellin than the application of control treatment.

Gibberellin is one of the plant growth regulators which has numerous and various effects on the growth and development of many plants. Also, it has been stated that the use of high concentrations of this hormone has increased the leaf growth index in some plants (Atri, 1996). In a study, it was observed that numerous compounds similar to gibberellin were found in isolated extracts of saffron corms and their amounts were varied in different stages of plant growth and development. The results of the mentioned study showed that the lowest amount of these compounds was observed in the sleeping corms and the highest amount of them was achieved in the flowering stage of the mother corms (Farooq and Koul, 1983). Chrungoo and Farooq (1984) treated big corms of saffron with different concentrations of gibberellin (100 to 500 ppm). After planting, they observed that flowering, the number of flowers, and the weight of produced flowers traits were increased with increasing gibberellin concentrations. Some studies also found that low levels of some growth plant regulators have many effects on growth, development, and yield of plants. These materials also affected many aspects of plant growth, such as flowering, rooting, and other processes (Shekari *et al*. 2005). Gibberellins regulate the plant growth by affecting the stem height, plant germination, and transition from vegetative to reproductive phase (Stephen *et al*., 2005). The use of gibberellic acid at a concentration of 10 mg.L with 3% sucrose increased the flower diameter in the cut rose flower (Emami *et al*., 2009). Arun et al. (2000) examined the effects of different levels of GA3 on the growth and flowering indices of the cut flower of rose "first red". They reported that GA3 can enhance the mentioned characteristics of this plant. Some studies have also shown that the application of different levels of GA in different plants had positive effects on some traits such as germination rate, plant height, flowering stem length, and flower diameter (Ahmadpour and Zarghami, 2009; Fakhraei Lahiji *et al*., 2011). The use of accel (combination of BA and GA417) at a concentration of 25 $mg.L^{-1}$ of benzyladenine caused a delay in flower aging, increased vase life, and increased the quality of post harvesting of cut alstroemeria flowers (Mutui et al., 2001). Arefnia et al. (2011) stated that leaf salting with a concentration of 100 mg.L^{-1} of bennyryl adenine on the oriental lily flowers produced the highest flower diameter. The use of accel (a combination of BA and GA417) at a concentration of 25 mg.L^{-1} of benzyladenine delayed the flower aging, increased the vase life, and increased the postharvest quality of cut alstroemeria flowers (Mutui *et al*., 2001). Arefnia et al. (2011) stated that leaf salting with a concentration of 100 mg.L^{-1} of benzyladenine on the oriental lily flowers produced the highest flower diameter. The use of accel (a combination of BA and GA417) at a concentration of 25 mg.L^{-1} of benzyladenine delayed the flower aging, increased the vase life, and increased the postharvest quality of cut alstroemeria flowers (Mutui *et al*., 2001). Arefnia *et al*. (2011) stated that the foliar application of benzyladenine under a concentration of 100 mg.L^{-1} increased the flower diameter in lily flowers.

Gibberellin enhances the cellular elasticity and reduces the water potential of the plant cell by concentrating the plant cell sap through hydrolysis the starch-to-sugar. Also, this plant hormone causes the entrance of more water to cells and cell elongation process (Stephen *et al*., 2005). Also, gibberellin increases plant growth and internode spacing by increasing the cell division (hyperplasia), cell size (hypertrophy), stem height, and the number of leaves (Arun et al., 2000). Some studies have also suggested that in some cases stem diameter index may be reduced under the application of gibberellin treatment, and probably this effect may be due to the role of this material in facilitating plant growth. Because, gibberellin affects the plant growth rate by stimulating and accelerating the cell division, cell elongation, and cell enlargement and decreases the stem diameter in numerous plants (Khangoli, 2001).

Esmaeili et al. (2013) showed that gibberellin reduced flower diameter, stem diameter, number of lateral stems, and number of flowers in *Zinnia elegans*. Gibberellin also reduced the time interval between transplanting and flowering stages and increased the vase life of the plant in farm and storage conditions.

Singh and Bijimol (2003) reported that the planting distance of 30 × 25 cm reduced the number of days to the emergence of flowering stem while increased the length and diameter of the inflorescence, the number of florets per inflorescence, the number of cut flowers per unit area, and the number of florets in the post-harvest stage. On the other hand, the studied treatment had no significant effect on vase life and the amount of water uptake indices of cut gladiolus flowers. Sharma and Talkudar (2003) determined the time interval × planting distance on corm formation and its yield of gladiolus. They showed that all growth indices, except the number of average corms, were higher than other treatments in the application of planting distance of 20 × 45 cm. Furthermore, the results of the mentioned study indicated that the number of average corms in planting distance of 25 × 60 cm was more than other treatments. The results of Singh *et al*. (2004) considered the effects of planting time, planting distance, and planting depth on growth and flowering indices of gladiolus cv "Sylvia". These researchers commented that planting distance of 45 × 20 and 65 × 25 cm had better effects on days to shoot emergence, inflorescence emergence, and opening the first floret compared to other treatments. Singh and Singh (2004) also investigated the effect of foliar application of zinc sulfate and planting distance treatments on growth indices and flowering stage of gladiolus. Above researchers indicated that planting distance significantly affected the number of leaves per plant so that the highest number of leaves was obtained in the planting distance of 20 × 25 cm. Nair and Singh (2004) investigated the effect of planting distance on flowering and growth stages of different cultivars of gladiolus and indicated that the distance of 30 × 25 cm was the best distance for the culture of gladiolus spp. Sharma and Gupta (2003) after studying the effect of corm size and planting distance on growth and flowering indices of gladiolus stated that increases planting distance resulted in increases plant height, length of flowering stem, and the number of florets per inflorescence. Also, the interaction between planting distance and corm size was significant on corm and cormel characteristics.

Moshrefi (1998) reported that there was no significant difference between studied plant densities and vegetative and reproductive indices of *Fritillaria imperialis*. On the other hand, they indicated that the stated traits decreased with increasing distance between plants.

Daneshvar and Heidari. (2009) examined the effects of plant density and planting pattern on growth and flower characteristics of gladiolus plant. Above researchers showed that the planting distance and interaction of planting distance × planting pattern had a significant difference in the flowering stem length of gladiolus cut flower. Also, Corbesier et al. (2003) reported that changes in the ratio of endogenous plant growth regulators affect the reproductive characteristics of plants. Accordingly, it is possible that changing the planting distance or planting pattern have changed the ratio of plant growth regulators released from the root by stimulating the root system. These changes can also change the inflorescence properties per plants.

Nowadays, the short longevity of leaves and cut flowers is one of the most important problems in the production of ornamental plants. Plant morphology and vase life of ornamental plants are important factors for assessing the flower quality (Iqbal et al., 2012; Ortiz et al., 2012); therefore, the application of methods that can increase the flower's life is significant (Schoellhorn et al., 2010). Accordingly, this experiment was conducted to determine the best concentration of gibberellic acid and plant density of tuberose in the winter production of flower in climate conditions of southern Kerman province. Therefore, we investigated the effects of plant density changes and treating the amount of gibberellic acid on growth and flowering indices of tuberose plant.

2. Material and Methods

In order to investigate the effect of plant density and treating the tuberose bulbs with gibberellic acid, an experiment was conducted using a split-plot based on the randomized complete block design in a research farm located in Jiroft city. In this experiment, planting density was considered as the main factor in 3 levels (10, 15, and 25 plants per m^2) and the concentrations of gibberellic acid as a sub-factor in four levels (10, 100, 200, and 300 ppm). The experiment was carried out in 12 treatments and 3 replications, which were consisted of 36 experimental plots.

Before planting, the bulbs with a diameter of 3-4 cm were selected and disinfected with sodium hypochlorite 1% for 5 minutes and washed with distilled water. The bulbs were then placed in concentrations of gibberellic acid for 5 hours. Also, studied bulbs were re-disinfected with the powdery fungicide of Zineb 1%. Prior to planting, the experimental farm was ploughed in the autumn season and fertilization process was carried out based on the recommendations of the Agricultural and Natural Resources Research and Education Center of Jiroft. Accordingly, NPK fertilizers (220-250-200 $kg.ha^{-1}$) were uniformly distributed with fertilizer resources of potassium (potassium sulfate), phosphorus (ammonium phosphate) and nitrogen (urea) in experimental plots. One-third of the nitrogen fertilizer was used simultaneously with the planting operation. On the other hand, two-thirds of the remaining nitrogen fertilizer were distributed in the form of top-dressing after planting in two stages of leaf formation and flowering. The bulbs were planted in the plots after being dried in the open air. In the present study, the bulbs were planted in the depth of 10 cm with the inter-row spacing of 30 cm and intra-row spacing based on the plant density (planting map). Irrigation operations were carried out based on the border irrigation with the brigade bar of super drip with holes of 10 cm interval. Weeding operations and control of the pests and diseases were applied in the same way for all treatments. Sampling was started at the flowering stage (mid-June). Then, some traits such as plant height, leaf length, number of leaves per plant, days to the appearance of the first inflorescence symptoms, number of florets per inflorescences, and number of offspring were evaluated at the end of the experiment.

2.1 Statistical analysis

The data were analyzed by SAS software, and the Duncan's Multiple Range test was used at $p<0.05$ to compare the mean of the data. The charts were drawn using Microsoft Excel software.

3. Results and Discussion

The results of the variance analysis of evaluated traits in tuberose plant indicated that plant density treatment had significant effects on plant height, the number of leaves per plant, leaf length, inflorescence length, and the number of florets per inflorescence were significant at $p<0.05$ under application of the mentioned treatment. On the opposite side, based on the results observed that plant density treatment had no significant effects on the number of cormels. On the other hand, the results showed that different concentrations of gibberellic acid had significant effects at $p<0.01$ on plant height and the number of florets per inflorescence and at $p<0.05$ on the leaf length and inflorescence length indices. Nevertheless, the application of the mentioned treatment was not significant for the number of leaf and cormel indices. In addition, the interaction of different concentrations of gibberellin hormone × plant height density was significant at $p<0.01$on inflorescence length trait and at $p<0.05$ on plant height, leaf length, and the number of florets per inflorescence. In addition to the above results, studying the interaction of treatments was not significantly different for the number of leaves and cormels (Table 1).

The results of the mean comparisons between studied treatments indicated that that the effects of gibberellin application on morphological traits were concentration-dependent so that the highest plant height (equal to 81.22 cm) was obtained in the interaction of 300 ppm of gibberellin × density of 15 plants per m^2. On the other hand, the lowest amounts of plant height (equal to 69.89 and 70.32 cm) in no application of gibberellin × density of 15 plants per m2 and no application of gibberellin × density of 25 plants per m^2, respectively. Based on the above results, it can be concluded that increasing the concentration of hormone may cause more cell division, and the suitability of plant density (15 plants per square meter) can increase plant growth during low intra-competition and appropriate distribution of nutrients among plants.

The maximum number of leaves per plant was related to the application of 100 ppm of gibberellin × 25 plants per m2 with an average of 20.55 leaves per plant and the maximum number of leaves per plant was obtained in no application of gibberellin × 10 plants per m^2. These results can be due to the gibberellin balance in this treatment so that the presence of equivalency in endogenous gibberellin may be prevented the conversion of vegetative phase to the reproductive phase, and consequently, the number of leaves per plant was increased.

The leaf length was influenced by the interaction of hormone × plant density so that the highest leaf length (with an average of 26.44 cm) was observed in the interaction of 300 ppm of gibberellin × 10 plants per m^2, and the lowest leaf length per plant was obtained in 200 ppm of gibberellin × 15 plants per m^2.

By increasing the concentration of gibberellin and increasing the distance between plants (decreasing the plant density in the studied tuberose plant) increased the number of corms. In general, the results revealed that the number of corms was reached to 1.66 corms per plant in the application of 300 ppm of gibberellin × 10 plants per m^2. Furthermore, the lowest number of corm (with an average of 0.44 corm per plant) was observed in the no application of gibberellin × 25 plants per m^2.

At the end, the highest number of florets per inflorescence (26.77 florets) was obtained in the concentration of 300 ppm of gibberellin × density of 15 plants per m^2 interaction. On the contrary, the lowest number of florets per inflorescence (equal to 20.66 florets) was observed in the interaction of no application of gibberellin × density of 25 plants per m^2.

4. Conclusion

The results of this study showed that all of the examined traits were affected by hormone application and plant density, except the number of onions per plant. In general, by application of gibberellin hormone under 300 ppm concentration and density of 15 plants per m^2 of tuberose bulbs were obtained more appropriate results than the other treatments. In this study, the highest amounts of plant height, number of leaves per plant, inflorescence length, and the number of florets per inflorescence were obtained in the 300 ppm of gibberellin × 15 plants per m^2. On the other hand, the lowest amounts in the above traits were observed in no application of gibberellin hormone × 25 plants per m^2. Therefore, the best treatment for the planting of tuberose corms was the use of gibberellin hormone under the concentration of 300 ppm and plant density of 15 plants per m^2.

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