An Investigation into the Effect of Gibberellic Acid and Storage Temperature on Vegetative and Reproductive Characteristics of Tuberose (*Polianthes tuberosa*)

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Tuberose (*Polianthes tuberosa* L.), from Agavaceae family, is one of the most important cut flowers in tropical and sub tropical areas of the world and has the seventh rate of production between all cut flowers of Iran. In order to investigate different effects of storage temperatures (8 weeks at 4°C, 4 weeks at 4°C + 4 weeks at 20°C, 8 weeks at 12°C, 4 weeks at 12°C + 4 weeks at 20°C and 8 week at 20°C) and different concentrations of gibberellic acid (0, 150 and 300 ppm) on quantitative characteristics of tuberose ‘Double’, a factorial experiment was carried out in a Randomized Complete Block Design with three replications. The effects of treatments were investigated on fresh and dry weight, height and number of flower stems, length of spike, speed of germination and flowering, weight and diameter of florets, number and weight of bulbs, and flower longevity. Qualitative and quantitative investigation of growth and flowering indices showed that the highest number of stems was recorded at 20°C storage temperature, which was 20% higher than that at 4°C storage temperature. Stem weight showed a significant correlation with most growth parameters. The most important effect of studied treatments was observed on growth speed. Speed of bulbs germination was affected only by storage temperatures (P<0.001), while higher GA3 concentration decreased both germination speed (P<0.05) and flowering speed (P<0.001). Finally, we suggested that the best treatment was storing bulbs for 4 weeks at 12°C + 4 weeks at 20°C before cultivation and GA3 treatment did not have any significant effects on growth factors of tuberose.

**Keywords:** Dormancy, Flowering, Germination, Gibberellic acid, Growth speed.
INTRODUCTION

Tuberose (Polianthes tuberosa L.) is a perennial plant, monocot, from Agavaceae family. This genus that is endemic to Mexico contains 14 species, 3 varieties, and 2 cultivars (Solano and Patricia Feria, 2007). Polianthes tuberosa is one of the most important cut flowers in tropical and subtropical areas of the world. P. tuberosa is the only species which is cultivated in most portions of the world. Tuberose is a bulbous plant, having white fragrant flowers (Hertogh and Nard, 1993). Tuberose flower is native to Mexico and then it was transferred to Europe and other parts of east lands. The name Polianthes has been derived from 'polis' which means white and 'anthes' which means flower (Hertogh and Nard, 1993). Tuberose flower is a rich source of essential oils with pleasant sweet aroma which has a wide range of applications in perfumery and cosmetics industry (Nagar, 1995; Rammamurthy et al., 2010).

In Iran 30'500'000 tuberose cut flowers were produced from 119 ha cultivation area. The average yield was around 26 stems per m² and the number of producers was around 175 people. The provinces of Khuzestan, Tehran and Markazi had first to third ranks of production. Polianthes tuberosa has seventh rank of production between all the cut flowers.

Bañón et al. (1997) and Nagar (1995) reported that storage temperature and different external conditions were effective on internal composition and physiological activities of bulbs during dormancy and subsequently during growth and flowering. It is believed that tuberose, as a tropical plant which is native to Mexico, is not capable of survival at lower temperatures (around 5°C). So, it never needs lower temperature during its life cycle including flower induction or natural growth.

Therefore, it is clear that the thermal treatment of 5°C and its duration are applied experimentally without an understanding of its physiological effects. It has been reported that endogenous gibberellin, which is induced by lower temperature, was increased and that it was related to the fast elongation in some geophytes, radish, carnation and aster. Also, exogenous gibberellin treatment could increase the length of flowering stem, the length of spike, and the number of tuberose florets (Huang et al., 1995).

Gibberellins stimulate the physiological responses of the plants and change the source-sink metabolism through influencing the photosynthesis and creating new sink organs in the plant. Studies indicated that signaling GA in keeping source-sink relation was related to the transfer of sucrose in phloem and far sink organs or tissues influenced overall performance or growth of the plants. Gibberelic acid treatment has important role in stimulating plant’s development process as early flowering increases the length or height of shrub, the number of leaves, the amount of chlorophyll and improves yield and quality in many of flowering plants (Rani and Singh, 2013).

Gibberellins are effective on increasing the length of flowering stem in cut flowers, accelerating flowering in a lot of bulbous plants, stimulating stem elongation and preventing flower bud abortion. These effects of gibberellin treatments might be associated with the increase in sink power by flowers when the daughter bulbs are competing for the storage material of mother bulbs and photosynthesis (Chang et al., 2001).

In an experiment, 150 ppm gibberellin was applied for treating tuberose bulbs before their cultivation and it increased plant height, the number of leaves, and the number of florets per inflorescence (Singh and Arora, 2000).

Most plant growth regulators do not act alone, but they will become effective in combination with other regulators. The effect of these compounds is related to their concentration. High concentration can inhibit the growth. This is very important for practical use in agriculture and also, its effect depends on application time and physiological conditions of the plant.

Our goals were increasing quality, uniformity and speed of flowering, determining best storage conditions and breaking dormancy of bulbs, and investigating the effects of temperature and GA₃ on growth and flowering of tuberose.

MATERIALS AND METHODS

This study was laid out in the Ornamental Plants Research Center (longitude of 35° 27' 30" E. and latitude of 33° 54' 30" N.). Bulbs of tuberose cv. ‘Double’ were procured from Dezful city,
Iran and then, similar bulbs with the same size were selected for cultivation. Finally, 40 bulbs with medium size were selected and cultivated with 20 × 20 cm distance in 1 × 2 m² plots in open space. The experiment was carried out in the form of factorial block design with three replications. Each block contained 15 plots and each plot contained 50 bulbs. In total, 1800 bulbs were cultivated.

In this experiment, five thermal treatments were investigated for storing fresh harvested bulbs, which included:

- 8 weeks at 4 °C
- 4 weeks at 4 °C + 4 weeks at 20 °C
- 8 weeks at 12 °C
- 4 weeks at 12 °C + 4 weeks at 20 °C
- 8 weeks at 20 °C

Different concentrations of gibberellic acid (0, 150 and 300 mg L⁻¹) were also prepared for dipping the bulbs before cultivation (for 5 minutes).

The effect of different treatments were estimated on different factors including fresh and dry weight, height and number of flower stems, length of spike, speed of germination and flowering, weight and diameter of florets, number and weight of bulbs, and vase life of cut flowers. The number of days from bulb cultivation to germination (speed of germination) and germination to flowering (speed of flowering) were calculated for all plants in each plot. The number of flower stems was counted in each plot. Mean of number and weight of bulbs were recorded for three plants at the end of growth season.

In order to evaluate the quality of flowers, they were harvested in early morning. Cut flowers were transferred to the laboratory of postharvest physiology to measure their different growth characteristics. The measured traits for three samples were averaged to be recorded for growth indices. For estimating cut flowers longevity, after transferring cut flowers to the laboratory, stems were re-cut in the water, disinfected with sodium hypochloride and then placed in distilled water at the room condition (22 ± 2 °C temperatures and 65 % relative humidity). The end of flower longevity was considered as the time when 50 % of florets were withered (Fig. 1.). In this stage, flower longevity which was affected by different treatments was recorded. All data were recorded by SAS Software Package and analyzed with Duncan test. Then, the figures were drawn by MS-Excel Software Package.

RESULTS AND DISCUSSION

Number of stems is affected by both storage temperature and GA₃ concentration (P<0.05) (Table 1). The highest number of stems was related to treatment with 20°C storage temperature and it was around 20% higher than that under the treatment with 4°C storage temperature (Fig. 2). It seems that increasing storage temperature increases the number of flowering stems. But, GA₃ treatment decreased the number of flowering stems. The extent of this decrease was 10 % in the treatment of GA₃ 150 ppm and around 22 % in 300 ppm concentration (Fig. 3). The number of stems showed significant correlation with the weight of stems and the weight and number of bulbs (Table 2). Also, higher number of stems was associated with lower flowering speed (P<0.001).

It has been reported that lower storage temperature did not have any effects on the produc-
tion of tuberose cut flower. As compared to lower temperature storage, storing at 25°C resulted in the loss of more water, but increased germination and flowering and improved the quality of cut flowers. So, tuberose bulbs should be stored at temperatures higher than 5°C for late cultivation (Huang and Okubo, 1995). Bhosale et al. (2014) also reported that storing bulbs for one month after harvest and dipping bulbs in 200 ppm of GA₃ before cultivation can improve growth and flowering characteristics. Gibberellic acid plays an important role in flower induction and in the stage when plant height is around 5 cm, the application of gibberellic acid can speed up flower induction in tuberose cv. ‘Single’ (Chang et al., 2001). An increase in GA₁ and GA₂₀, and a decrease in GA₁₉ levels, was reported to coincide with the transition from the vegetative phase to the stages of early floral initiation and flower development. GA₅₃ stayed at constant levels at the vegetative, early floral initiation, and flower development stages. The absence of GA₁ in vegetative corms and its presence in corms at early floral initiation and flower development stages suggest that GA₁ is a causal factor in inducing floral initiation in P. tuberose (Chang et al., 2001). The negative effect

Table 1. Analysis of variance for studied traits.

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<td>GA₃</td>
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<td>79.184</td>
<td>135.214</td>
<td>8.684</td>
<td>1.575</td>
<td>246.0</td>
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<td>T × GA₃</td>
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*Note: *, **, *** = Significance at 0.05, 0.01 and 0.001 levels and non-significance, respectively.

Table 1. Continued.

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<td>7.022</td>
<td>587.489</td>
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<td>0.976</td>
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<td>8.02</td>
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</table>

*Note: *, **, *** = Significance at 0.05, 0.01 and 0.001 levels and non-significance, respectively.

Fig. 2. Effect of storage temperature on the number of flowering stems.
Fig. 3. Effect of GA₃ concentration on the number of flowering stems.

*Similar letters show non-significant differences at the 5% level of significance on the basis of Duncan test.
of GA₃ on the number of flowers in this study is likely to be related to the type or time of its application before cultivation.

Stem weight was also affected by storage temperature (P<0.05) and GA₃ concentration (P<0.01) (Table 1). The highest stem weight was recorded at 20°C storage temperature and the lowest one at 12°C storage temperature (Fig. 4). As GA₃ concentration was increased, stem weight was decreased (Fig. 5). Stem weight showed significant correlation with most growth characteristics such as the length and dry weight of stems, the length of spike, the weight and diameter of florets, and the weight of bulbs. As well, the increase in flowering speed as occurred in most storage with higher temperature treatments resulted in lower stem weight.

According to results, simple effect of time and GA and their interaction were significant on the length of flowering stem. Obviously, the length of stem was correlated with stem weight (P<0.001) (Table 2). The length of spike as a qualitative factor has often attracted the attention of the producers and buyers of tuberose flowers. Our results were not in agreement with the findings of Mukhopadhydy and Bankar (1983) who reported that 75 ml/L of GA₃ increased the length of flowering stem, the number of florets, and the length of inflorescence in tuberose cv. ‘Single’.

Our results were inconsistent with the findings of Jana and Biswas (1982) and Abbasi (2009). It has been proved that GA₃ plays the main role in increasing the length of flowering stem in many plants and its concentration is different in several plants. Our results are in agreement with the findings of Prreeti and Gogoi (1997) that showed that the increase in the concentration of gibberellic acid and benzyl adenine had positive effect on increasing length the of tuberose flowering stem, cv. ‘Single’.

Also, Kumar et al. (2011) showed that higher concentration of gibberellic acid was associated with better quality traits of flowers. They explained that stimulatory effect of GA₃ on growth factors can be related to its effect on the transfer of compounds and elongation of cells which finally improved flowering characteristics.

But, some researchers believe that applying gibberellic acid on chilled and rootless bulbs will also stimulate the growth of internodes and will finally induce flowering (Shillo, 1992). It seems that the effect of GA₃ treatment on bulb had different effects as compared to GA3 spray on the plants and its result will be clear in increasing the length of shoot after flowering and formation of flowering stem. About the effect of GA₃ foliar spray, Sable et al. (2015) reported that the highest amounts of qualitative factors including the height of the plant, the number of leaves and leaf area were recorded when GA₃ concentration was increased.

In case of black Iris (Iris nigricans Dinsm.), it has been reported that the effect of GA₃ on characters of flowering stem was related to application method and its concentration. Plants irrigated with 1 mg/L GA₃ produced the longest and heaviest flowering stems. On the other hand, flowering was delayed (200 days) and stems needed more time for receiving photosynthetic com-
pounds from the leaf, whilst plants sprayed with 375 or 500 mg/L GA$_3$ showed the lowest and lightest flowering stems because flowers opened (160 day after cultivation) before all the treatments (Al-khassawneh et al., 2006).

Totally, it seems that the effect of GA$_3$ on bulb differs from its foliar application on the plant and its effect on increasing length of stem appeared after flower induction and formation of flowering stem. For this reason, the results of this experiment for the effect of GA$_3$ on qualitative characters of flower were different from other findings.

The length of spike was only affected by storage temperature (P<0.05) so that the highest length of spike was related to 20°C storage temperature and the lowest one was recorded at 12°C storage temperature. Indeed, the difference between the highest and lowest amounts was 15% (Fig. 6).

The weight of floret had significant relation with storage temperature (P<0.05) and was affected by different treatments (Fig. 7). Floret weight also showed relationship with the weight of shoots and spike length (P<0.001) (Table 2).

None of the treatments had significant effect on floret diameter. But, this factor showed positive correlation with shoot weight and bulb weight at the 1% and 0.1% levels of significance, respectively.

Akbari and Tehranifar (2009) showed that 25°C during 4 and 8 weeks was the best condition for storing tuberose bulbs. When bulbs were stored for 8 weeks at 4°C, only few plants went to flowering phase which had very low quality.

Ehlers et al. (2003) reported that amount different thermal treatments that they applied on Veltheimia bracteata, 15 – 30°C storage temperature delayed germination of the bulbs and 30°C storage temperature accelerated it, but it damaged around 50% of the bulbs. They also expressed that the stored bulbs start flowering at 15°C, but the quality of flowering, the length of flowering shoot, and the number of floret were decreased.

We found that gibberellic acid treatment did not have any effects on the length of spike. Our results are in disagreement with the findings of Rani and Singh (2013). It seems that the effect of GA$_3$ was highly dependent on its concentration and the physiological conditions of the bulbs and even the balance of the hormones.

Flower longevity was not influenced by the studied factors. But, the correlation between flower longevity and the number of shoots was significant (P<0.05) (Table 2) and flower longevity was decreased with the increase in the number of flowering stems.

One of the most important and commercial uses of gibberellins is preventing postharvest yellowing, especially in monocots. The findings showed that after treating tuberose bulbs with GA (100 mg L$^{-1}$) and sucrose (4%) for 24 hours, storage life and opening florets were increased and production of ethylene was decreased. Finally, the quality of tuberose flowers were improved (Di-
Our results are consistent with the findings which showed that 100 ppm of GA3 significantly increased longevity of tuberose flowers. These results confirm the other findings about tuberose and tulip (Nagarja et al., 1999; Prreeti and Gogoi 1997; Rudnicki et al., 1976).

Fresh weight and number of produced bulbs did not exhibit any relationships with the storage temperature and gibberellic acid treatment. Our result are opposite with the results of Abbasi (2009) which showed a negative linear relation of different concentrations of gibberellic acid with the number of produced bulbs so that as the concentration of gibberellic acid was increased, the number of bulbs was decreased.

By reducing plants vegetative cycle with gibberellic acid, bulbs and new buds were induced. Gibberellic acid increased the number of bulbs in tuberose flower (Ram et al., 2002). On the other hand, increasing concentration of gibberellic acid not only could not increase the number of bulbs, but it also decreased its number. There is a probability that 50 ppm gibberellic acid can induce assimilates in the bulbs and keep up the amount of water potential to increase the number of new bulbs. But, higher concentrations showed inhibitory effect. Our results showed that thermal treatment and gibberellic acid did not have any significant effects on the increase in bulb yield in tuberose, which is in agreement with the findings of Al-khassawneh et al. (2006) who showed that gibberellic acid did not have any effects on the number of suckers in black iris (Iris nigricans Dinsm.) plant.

The most important effects of the studied treatments were on the speed of growth characteristics. Storage temperature was only effective on the speed of germination in bulbs (P<0.001), while GA3 concentrations were also effective on the speed of germination (P<0.05) and the speed of flowering (P<0.001) (Table 1). With the increase in storage temperature, the speed of germination was also increased, but this treatment had no effects on the speed of flowering. On the other hand, the speed of flowering was completely affected by GA3 concentration, and the time required from bulb germination to flowering was increased with the concentration of GA3 solution (Fig. 8). The speed of flowering as affected by GA3 solution treatment with 150 ppm concentration was around 6% less than control and this decrease was around 13% in 300 ppm concentration. Banan et al. (1997) and Nagar (1995) reported that storage temperature and different external conditions were effective on internal compounds and physiological activities of bulbs during dormancy and subsequently, on growth and flowering. In other studies the effect of 4, 10 and 30°C of thermal treatments was investigated on tuberose flower for 10, 20 and 30 days and it was concluded that different conditions of storage was significantly effective on germination, growth and flowering. Finally, it was reported that 10°C during 30 days is most effective treatment on improving growth and flowering (Dhua et al., 1987). Since storing tuberose bulb for a long time in room temperature dries and reduces the number of bulbs, so, commercial bulbs are stored at 5°C. But, induction and development of flowers did not happen in this condition and it was started after cultivation. Delay in germination and flowering, and even no-flowering, was observed by increasing storage duration at both 5 and 25°C storage temperature (Huang et al., 1995). As the speed of flowering increased, most qualitative characteristics of the flower such as the number and weight of flowering stems (P<0.001), the dry weight of shoot, the weight of floret and flower longevity (P<0.05) were decreased (Table 2).

It has been reported that the amount of gibberellic acid was higher in large size bulbs of tuberose and the amount of GA3 might have been related with faster flowering in these bulbs. 5°C Led to an increase in gibberellic acid activity in the bulbs compared to 25°C. This shows that in tuberose flower, there is no direct relationship between higher gibberellic acid activity induced by low storage temperature and growth after cultivation (Huang et al., 1995). It seems that low temperature during bulb storage does not have any effects on flower induction before and after cultivation, or forcing maturity in tuberose flower. This is opposite to the role of low temperature in
other tropical geophytes such as *Hippeastrum hybridum* which is like tuberose endemic to Mexico. Storing bulbs of *Hippeastrum hybridum* at the temperature of lower than 13°C for 8 - 10 weeks or at 5 - 9°C for longer duration accelerated the flowering (Huang and Okubo, 1995). In another study, the effect of different thermal treatments including 15, 20 and 25°C with different durations including 2, 3 and 4 months was studied on germination, flowering and amount of carbohydrates in ornamental ginger and it was concluded that extending storage duration, especially at higher temperatures, delayed germination and decreased flowering (Paz et al., 2003). In the commercial conditions, tuberose bulbs should be stored for 3 months at 4 - 5°C and then, they should be planted. Nonetheless, gibberellic acid pretreatment did not have any effects on breaking dormancy and germination (Nagar, 1995). The application of gibberellic acid along or along with benzyl adenine (100 mg L⁻¹) advanced the appearance of flowering stem and induced flowering (Kioshi, 2003). Sajjad et al. (2015) also reported that dipping corms of gladiolus in benzyl adenine improved qualitative factors of gladiolus flowers.

Between all gibberellins, GA₃₂ was the most effective compound for flower induction in tuberose flower. In the other hand, GA₁, GA₃₃, GA₄ and GA₂₀ had a very low effect on the formation of flowering stem in tuberose (Chang et al., 2001). However, tuberose cut flowers have been cultivated in a large area, but there are no documents showing the best storage conditions.

![Fig. 8. Interaction of storage temperature and GA₃ concentration for the speed of germination and flowering in tuberose flower.](Image)

Table 2. Correlation coefficients of studied traits.

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*, **, ***; ns = Significance at 0.05, 0.01 and 0.001 levels and non-significance, respectively.

The role of gibberellins in the time of flowering in different plant species varied. Totally, GA application can be an alternative for both factors of long days and low temperatures. Moreover, it has been reported that GA4/7 is suitable for flowering stimulation in pine species (Pinaceae). It means that it influences the plants whose flowering is not affected by day length. Also, it has been seen that gibberellins can stimulate flower induction in some ornamental plants without reacting to photoperiod and cooling (Chang et al., 2001).

GA3 contains a group of carboxyl in carbon No. 7 and two groups of hydroxyl which are related to its activity. Any changes in the position of hydroxyl group can enable or disable GA3. Furthermore, the application of exogenous gibberellins cannot release auxiliary buds from apical dominance. But it can cause fast growth of buds which are free of apical dominance (Al-khassawneh et al., 2006).

Literature Cited


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